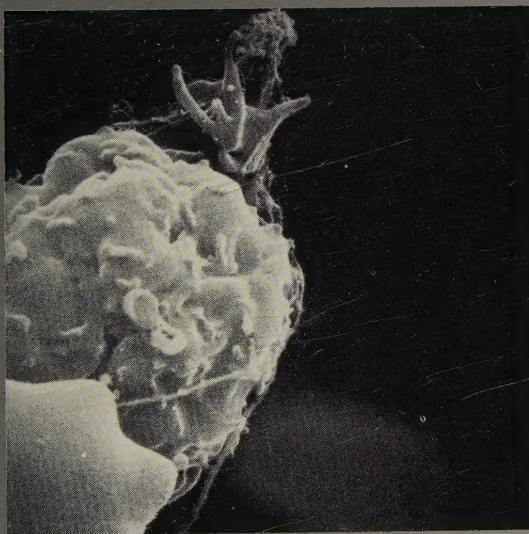


lund 1983

Jan B. Wieslander

MICROARTERIAL ANASTOMOSES

*Experimental studies on blood volume flow,
luminal area, platelet accumulation and repair processes*



Malmö 1983

MICROARTERIAL ANASTOMOSES

Experimental Studies on Blood Volume Flow, Luminal Area,
Platelet Accumulation and Repair Processes

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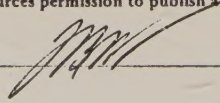
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Title and subtitle MICROARTERIAL ANASTOMOSES. Experimental studies on blood volume flow, luminal area, platelet accumulation and repair processes.			
Abstract			
Microarterial anastomoses were performed in rabbits on the central arteries of the ear and saphenous arteries (0.8-1.2 mm), femoral arteries (1.7-2.0 mm) and renal arteries (2.0-2.7 mm). Experimental methods were developed for studying blood volume flow (electromagnetic flowmetry) and platelet accumulation (tracer technique using 32 P labelled platelets) enabling continuous in vivo registration of these parameters following anastomotic procedures in small vessels. Two anastomotic techniques, conventional end-to-end and end-in-end (sleeve) anastomotic techniques, were thus studied and compared regarding blood volume flow, luminal area, platelet accumulation and repair processes. The conclusions were: 1) Blood volume flow (vessel diameters 0.8-1.2 mm) was not significantly altered following end-to-end anastomosis but following end-in-end anastomosis a reduction by 50% up to 3 days postoperatively was registered. 2) The luminal area (vessel diameters 0.8-1.2 mm) was slightly reduced only at 1 hour postoperatively following end-to-end technique. The end-in-end technique reduced luminal area to 25% of the normal immediately postoperatively followed by a gradual increase to 65% three months postoperatively. The reduction in luminal area was less severe using end-in-end technique in larger arteries (femoral, renal). 3) Accumulation of platelets was more pronounced following end-to-end technique than following end-in-end technique. 4) Luminal narrowing using end-in-end technique depends mainly on the doubling of the vascular walls. The gradual increase in luminal area depends mainly on atrophy of the medial layers. 5) The repair processes following both techniques included neo-intimal hyperplasia inside fibrotic medial and adventitial layers. Re-endothelialization was completed between 7 and 14 days, using both techniques, but the endothelial sheet frequently remained abnormal up to 3 months postoperatively.			
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Date 1983-05-05

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MICROARTERIAL ANASTOMOSES

**Experimental studies on blood volume flow, luminal area,
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Malmö 1983



Legend to cover:

An activated platelet, projecting pseudopods, is just being caught at the top of a platelet thrombus. Scanning electron micrograph. x 12 000.

ABBREVIATIONS

ETE: end-to-end anastomosis

EIE: end-in-end, also called telescope or sleeve anastomosis

ETS: end-to-side

EMF: electromagnetic flowmetry

SEM: scanning electron microscopy

MBq: megabecquerel

GM tubes: Geiger-Müller tubes

PRP: platelet rich plasma

PPP: platelet poor plasma

The thesis is based on the following papers:

- I. Blood flow in small arteries after end-to-end and end-in-end anastomoses: an experimental quantitative comparison.
Wieslander JB and Åberg M.
J Microsurg 2:1121-125, 1980.
- II. Stenosis following end-in-end microarterial anastomosis: An angiographic comparison with the end-to-end technique.
Wieslander JB and Åberg M.
J Microsurg 3:151-155, 1982.
- III. Accumulation of isotope labelled platelets in small arteries after end-to-end and end-in-end anastomoses in the rabbit.
Wieslander JB, Åberg M and Dougan P.
Brit J Plast Surg 35:158-162, 1982.
- IV. A histological comparison of experimental microarterial end-in-end and end-to-end anastomoses.
Wieslander JB and Rausing A.
Plast Reconstr Surg. Accepted for publication.
- V. Endothelialization following end-to-end and end-in-end microarterial anastomoses. A scanning electron microscopical study.
Wieslander JB, v. Mecklenburg C and Åberg M.
Scand J Plast Reconstr Surg. Accepted for publication.

The publications are referred to in the text by their Roman numerals.

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INTRODUCTION

The history of reconstructive vascular surgery starts in the latter part of the last century. In earlier times, ligation of blood vessels - as described by Ambroise Paré (1517-1590) - was the only method used to treat bleeding vessels. Only a few attempts to repair damaged vessels have been reported (Hallowell, 1759). Successful experimental vascular anastomosis was first reported about one hundred years ago by several surgeons (Eck, 1879 and von Hirsch, 1881) and this inspired a considerable amount of research during the following 20 years. Just before the turn of the century, J.B. Murphy (1896) (133) performed the first successful arterial anastomosis in a patient with a bullet wound in the groin damaging the femoral artery. It is interesting to note that the vessel ends were united by an invagination technique. The foundations of modern vascular surgery were laid by Carell (40,41) & Guthrie (26,77,78). However, little progress was made in vascular surgery until the Korean War when the repair of large vessels became clinical routine.

Microsurgery is defined as surgery performed under magnification using a microscope. The first reports of the use of a microscope (monoscope) in the operating theatre date back to 1921 and the Swedish otolaryngologist Nylén.

In 1961, O. Eiken (59) reported basic studies in the reconstruction of small arteries in the dog using different grafts. Among his conclusions was that autologous veins are the only material suitable for replacing small arteries.

Early attempts to unite small blood vessels met with considerable difficulties, since suitable microinstruments and sutures were not available. With such necessary aids and performing anastomosis using an operation microscope (diploscope), Jacobson & Suarez (1960) (99,101) were the first to report 100% patency rate in anastomosing carotid arteries (diameter 1.4-3.2 mm) in rabbits and dogs. Microvascular surgery was introduced and enormous improvements in microscopes, microinstruments and microsutures have been made during the last two decades (2,3,7,

37,148,151,157). The development of low-pressure microvascular single and double approximator-clamps (8,89) minimizing the risk of vessel damage and allowing vessels to be turned has been of major importance (150,203).

Microsurgical techniques are now used in nearly all fields of surgery. Severed digits (31,34,36), limbs (16,47), and avulsed scalps (129,145) can be reimplanted and flaps of skin (53,54,85, 104), muscle (86,155), bone (35,182, 212,214,215,218), omentum (33) and digits (31,34,36,49,68,110) can be transferred (17,84, 87,99,114,152,176,177). In neurosurgery (130,131,149, 181), microsurgical techniques are used in extracranial-intracranial by-pass operations (207,208). Similar techniques are used in coronary by-pass operations in cardiac surgery. In orthopedic surgery, the severely damaged lower leg with skin, muscle and bone defects was previously a difficult problem often ending in amputation of the limb. Microvascular surgery has changed the outlook by permitting transfer of vascularized free flaps. The new cover with its good blood supply counteracts infection and allows the fracture to heal.

The scope of experimental microsurgery is constantly widening. Small animals are used for developing new operative techniques and studying organ transplantations (15). The advantages are obvious: procedures are ethically more acceptable and simplified and time-saving methods can be combined with a resulting dramatic reduction in cost. Microvascular experimental models have also become standard in testing new pharmacological agents, e.g., against rejection in organ transplantation.

The history of microvascular anastomoses

End-to-end anastomosis

The basic concepts of microvascular anastomosis date back to the ETE anastomoses in large vessels suggested by Carrel (1902) (26,40,41). However, the running sutures used in large vessel surgery have been avoided in microsurgery following observations

of narrowing at the anastomotic site (46). The basic steps advocated by Carrel using soft vascular clamps, resection of superfluous adventitia and guide sutures placed 120° apart are still in use. In 1967, Cobbet stressed the eccentric biangulation technique (50,51) which decreases the risk of including the opposite vessel wall in a suture; some microsurgeons use a temporary intraluminal stent to avoid this (142). The conventional ETE microvascular technique is well described in textbooks (10,32,45,46). The ETE technique is frequently combined with the use of vascular grafts (22,39,55,69,74,88,91,94,100,156,161,193,198,209).

End-to-side anastomosis

The ETS anastomosis has been in use for a long time in vascular surgery. The main indication for using this anastomotic technique is that the donor vessel cannot be transected because it feeds tissue of vital importance. Such a clinical situation is, e.g., the lower leg with only one remaining major artery (73). The ETS technique was a necessity for microsurgeons in performing extracranial-intracranial bypass (207,208). The optimum angle of union between the two vessels was earlier much debated. A small angle has been recommended, but later blood volume flow studies have shown that there is little difference between ETE and ETS. The degree of angle is probably of less importance than was previously thought (29,79,95,139,160).

End-in-end (sleeve) anastomosis

J.B. Murphy 1896 and Bouglé 1901 restored anatomical continuity between arterial ends using an invagination technique. In 1978, Lauritzen (115,116,117,118,119) and Meier (127) introduced the EIE technique in microvascular surgery. Both methods place sutures so as to avoid introducing foreign material into the vessel lumen. The placing of sutures in Meier's technique also preserves longitudinal tension in the telescoped segment. The goal has been to introduce quick and simple methods with patency rates comparable to those of conventional ETE anastomoses (48,111,113,143,174).

Non-suture (mechanical) anastomosis

Non-suture anastomosis can be performed using staplers or ring pins (42,43,96,199). Staplers cannot be used in microsurgery because the vessel wall must be everted 180° . The ring pin instrument was introduced by Nakayama (1962) for small vessel anastomosis. The vessel wall must be everted 90° , but this allows the method to be used down to vessels of 1.5 mm external diameter. In 1967, Bellman et al. (19) reported on the use of Androsov's (11) and Nakayama's apparatuses (135,136,137) and stated that excellent apposition of the intimas is one advantage using mechanical devices. Östrup 1976 (213,216,217) and later Lidman and Östrup 1981 (120) have reported excellent results obtained using Nakayama's ring pin device in venous microvascular anastomoses (200). With ring pins, the principal advantages are rapid and simple use, good apposition of intimas and no intraluminal suture material (211).

Suture material

Until recently, the suture material most widely used was synthetic non-absorbable monofilament thread (perlon, nylon, ethilon). In 1980, Mii et al. (128) compared absorbable and non-absorbable microsutures and reported more rapid reendothelialization following the use of polyglycolic acid than with nylon and polypropylene sutures. In 1981, Kragh (112) compared polypropylene and polyamid sutures (supramid and nylon) and found decreased thrombus formation using polypropylene. Absorbable suture materials seem to give less tissue reaction than synthetic nonabsorbable monofilament sutures. At present, there seems to be a tendency to prefer absorbable sutures of polyglycolic acid or polydioxanone.

VASCULAR ENDOTHELIAL DAMAGE

Normal endothelium

Normal vascular endothelium (141) is the only "blood compatible surface" known. Physical properties and biochemical events on the surfaces of endothelial cells protect against thrombus formation at the intima. Endothelial cells release the vasodilating and antiaggregatory prostaglandin PGI_2 (Prostacyclin) (30) and simultaneously the vessel wall produces plasminogen activators causing fibrinolysis of thrombi. It has recently been proposed that the balance between the proaggregatory precursors of prostaglandin, the endoperoxydes PGG_2 - PGH_2 and the antiaggregatory PGI_2 , controls the degree of thrombogenicity existing at the luminal surface (132). Whereas the concentration of the antiplatelet factor, PGI_2 , increases coming from the external to the internal layers of the vessel wall, that of the proaggregatory PGG_2 and PGH_2 decreases (27,28). Furthermore, the endothelial cells are themselves negatively-charged and repel platelets (164).

Endothelial damage

Exposed subendothelial layers induce changes in platelet morphology (92,192) and the release of ADP and the potent proaggregatory and vasoconstricting prostaglandin TxA_2 (171). Platelets thus adhere instantaneously to damaged areas, this being followed by aggregation (13,20,21,183,188,191,194,201,204,205). Platelet activation also leads to exposure of platelet factor 3 (PF3), which converts prothrombin to thrombin and leads to the formation of fibrin, which stabilizes the platelet thrombus (58, 109,154). Simultaneously, the production of the proaggregatory substances PGG_2 and PGH_2 in the vessel wall accelerates the formation of a platelet thrombus. Following intimal damage in small arteries, this initial thrombus formation rapidly reaches a maximum after reestablishing blood flow. The reversal of the process is due to the increasing release of PGI_2 from the intima resulting in cessation of platelet aggregation and, in

increasing concentrations, disaggregation of the platelet thrombus (60). Simultaneously, thrombus resolution by fibrinolysis is stimulated by release of plasminogen activators from the vessel wall (76,97,98,144,163).

Inspection of the intraluminal surface 1-2 hours after damage reveals a carpet of flattened platelets forming a new, temporary intima (140). Larger defects in the vessel wall are sealed by platelet aggregates and to a minor degree fibrin and erythrocytes (5,6,173).

Endothelialization

Complete neo-endothelialization occurs in small arteries and veins at 1-2 weeks post trauma (18,83,140,165,169,185). There are three hypotheses regarding the origin of endothelial cells:

- 1) Per continuitatem regeneration by cell division from undamaged areas (66,165),
- 2) blood-born genesis of cells (monocytes, fibroblasts, polymorphonuclear leucocytes) (71) and
- 3) smooth muscle cells migrating through the fragmented internal elastic lamina (paper V, ref 24).

The sub-endothelial part of the intima heals by the formation of fibrous intimal hyperplasia (187,195). In small animals, this process seems to reach a maximum 2-4 weeks postoperatively, but total occlusion of the vessel sometimes is the end result. The deeper layers (media and adventitia) frequently undergo necrosis and atrophy resulting in severe fibrosis (107,108). The internal and external elastic laminae remain discontinuous following transection of vessels. The development of abnormal endothelium, fibrous intimal hyperplasia and disintegration of the medial and adventitial layers can be caused by physical and/or chemical damage to the vessel wall (109).

PROBLEMS IN MICROVASCULAR SURGERY

Early and late anastomotic failures still constitute a greater problem in microvascular surgery than in surgery on larger vessels. Numerous problems, mainly related to the narrow vessel lumen and low flow states, particularly in veins, might result in excessive thrombus formation and decreased blood volume flow ultimately leading to inadequate organ perfusion. Numerous investigations have evaluated microvascular techniques, as well as the benefits of pharmacological agents by simple patency tests (4), ex vivo perfusion models, histopathology at intervals (9,18) or flap-survival models. Few attempts have been made to develop methods and experimental models enabling in vivo quantification of blood volume flow changes and of thrombus formation in microvascular surgery (5,6).

New microvascular techniques are constantly being developed, as well as new suture materials, antiplatelet drugs (61,62,122,191, 147), anticoagulative (63) and fibrinolytic agents. The effects from local application of spasmolytica (24,179) and administration of other blood flow promoting agents (106,200,210) is intensely discussed. Whether or not these innovations are advantageous have been difficult to evaluate. Thus, there is considerable need for in vivo methods enabling analysis of basic events such as blood volume flow and platelet thrombus formation following vascular anastomosis or damage to vascular endothelium in small-sized vessels. Such methods are a prerequisite for the correct evaluation of new microvascular methods and pharmacological antithrombotic agents, as well as of procedures now in use (56).

AIMS OF THE INVESTIGATION

The purpose of the present thesis was to develop methods for examining basic properties of microarterial end-to-end and end-in-end anastomoses by studying:

- blood volume flow during the first three days post anastomosis,
- anastomotic luminal area in vessels of different diameters during the first three months,
- thrombogenicity by registration of platelet accumulation in the anastomotic area up to four hours post-anastomosis,
- repair processes in the vessel walls and the transformation of platelet aggregates at anastomotic sites and
- the process of reendothelialization.

METHODS

Anesthesia:

All rabbits were anesthetized with intermittent injections of sodium pentobarbital (Mebumal Vet., 60 mg/ml, ACO Läkemedel AB, Solna, Sweden) in a marginal vein of the ear or a vein of the hind leg.

Temperature regulation:

The temperature of the animals was maintained at $39,8^{\circ}\text{C} \pm 0,2^{\circ}$ by a thermostatically-regulated lamp and an electric heating pad.

Irrigation:

The wound area was kept moist by irrigation with pre-warmed Tyrode's solution without MgSO_4 (NaCl 116.3 mmol, KCl 5.37 mmol, CaCl_2 6 H_2O 0.91 mmol, NaH_2PO_4 $2\text{H}_2\text{O}$ 0.95 mmol, NaHCO_3 26.2 mmol and glucose 5.56 mmol. The solution was buffered with sodium biphosphate 144 mg/l and sodium bicarbonate 2.2 g/l).

Prevention of vasospasm:

Small quantities of lidocaine (Xylocain 10 mg/ml, AB Astra, Södertälje, Sweden) were applied locally to prevent vasospasm.

Preparation of vessels:

The rabbit ear was fixed to a plastic mould. A skin flap measuring 3 x 2 cm was raised over the central vessels of the ear. The vein was ligated and the artery isolated with ligation of side branches using microsutures. In radioactivity studies, cartilage and skin below the prepared vessel were excised. EMF probes and GM tubes were fixed to the plastic mould.

Access to the femoral and saphenous arteries was made through skin incisions in the groin. Renal arteries were reached through an abdominal incision. Side branches were ligated as above.

Laboratory investigations:

Hemoglobin, hematocrit and platelet counts were followed in paper I + III.

Suture material:

In all cases, synthetic monofilament non-absorbable polyamid (Dermalon 10-0, Davis & Geck) on atraumatic tapers MV-140 was used.

EIE anastomosis:

An Acland double microvascular clamp was placed on the artery. The vessel was transected and superfluous adventitia excised for distances of 1-2 mm at each end of the cut vessel. Three stay sutures were placed at 120° intervals and a minimal number (3-5) of interrupted sutures placed among them.

EIE anastomosis:

Anastomosis was performed according to the techniques of either Lauritzen or Meier. In both cases, adventitia was excised for a distance of approximately 2 mm from the proximal vessel end and 1 mm from the distal vessel end.

Lauritzen technique:

Two sutures were placed 180° apart. Each suture was begun by inserting the needle through the distal vessel wall close to the end and then into the proximal vessel wall 2 mm from its end; only adventitia and part of the media were included in the pass through the proximal vessel. After tying the sutures, the proximal vessel stump was inserted into the distal vessel lumen. To prevent leakage, one or two sutures had often to be placed extraluminally midway between the first two sutures (Fig. 1).

Meier technique:

Two sutures were placed on opposite sides of the vessel. The needle was passed from the outside through the distal vessel wall 2 mm from the end and continued with a partial bite close to the end of the proximal vessel. The suture was completed by passing the needle through the distal vessel lumen and out through the wall close to the entry point. By tying the sutures, the proximal vessel stump can be pulled inside the distal lumen. Additional sutures can be placed at the junction between the cut end of the distal vessel and partially in the wall of the proximal vessel.

EIE

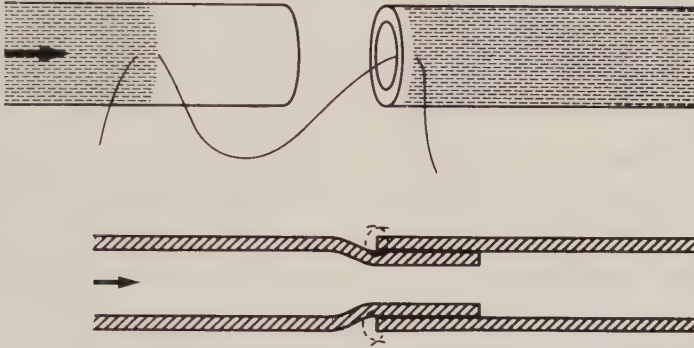
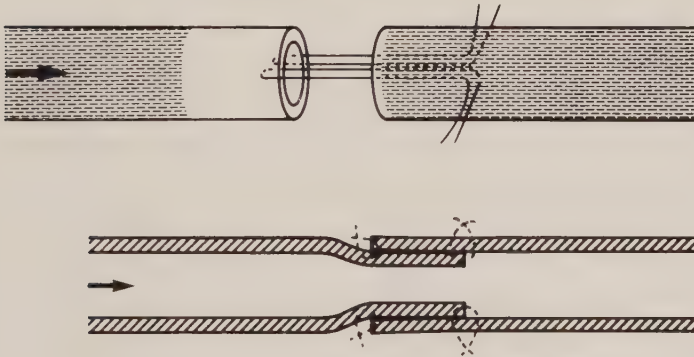
Ad modum LauritzenAd modum Meier

Fig. 1. EIE anastomoses according to the technique of Lauritzen (above) and Meier (below). Arrows indicate direction of blood flow.

Patency test:

The vessel was gently obstructed distal to the anastomosis with a pair of forceps. Using another pair of forceps, the vessel was emptied towards the anastomosis. Patency was indicated by refilling of the emptied vessel segment.

EMF:

Electromagnetic flowmetry was performed using a Blood Flow Meter 376, Nycotron AS, Oslo, Norway. Electromagnetic flow probe sizes were 1.0 and 1.2 mm.

Angiography:

- A) To perform angiograms of the central artery of the ear, a catheter was placed intraarterially several centimeters proximal to the anastomosis or in a side branch.
- B) To perform angiograms of the renal arteries, a catheter was inserted through the left femoral artery and positioned in the aorta at the origin of the renal arteries.
- C) To perform angiogram of the femoral and saphenous arteries, a catheter was inserted through the contralateral femoral artery with its tip at the bifurcation of the aorta.

Vasodilatation was obtained by infusion of Xylocain 10 mg/ml 0.5-1.0 ml shortly prior to angiography.

Primarily enlarged angiograms were obtained in vivo using projection angiography.

Contrast media: Urografin (Schering AG, Berlin, West Germany) - paper I.

Isopaque 350 mg I/ml (Nyegaard & Co., AS, Oslo, Norway) - paper II.

External diameter of (dilated) vessels: All vessels were dilated by topical application of small amounts of lidocaine (Xylocaine 10 mg/ml). Three measurements of the external vessel diameter were performed using a specially constructed pair of callipers. The mean value of three measurements was registered.

Vessel wall thickness: The external diameters of dilated vessels were measured as described above. Following excision, the vessel segment was thread over a tapered catheter section until the correct external diameter was reached. The corresponding internal vessel diameter could then be measured on the appropriate tapered catheter section.

Vessel wall thickness =

$$\frac{(\text{external vessel diameter}) - (\text{internal vessel diameter})}{2}$$

Luminal area:

The luminal areas of the anastomoses were calculated as percentages of the luminal areas of the "normal" vessel (see below) using the formula: $L = D_a^2 / D_v^2 \times 100$.

L = percentage of residual luminal area of the anastomosis.

D_a = smallest inner diameter of the anastomosis. D_v = inner vessel diameter, calculated as the mean value of diameters measured within 2 cm proximal and distal to the anastomosis and in a segment of the artery where the vessel calibre was uniform for at least 1 cm.

Isotope:

The beta-emitter ^{32}P (half-life 14.3 days) was used as the orthophosphate in dilute hydrochloric acid (pH 2-3).

Platelet labelling:

100-150 ml of homologous whole blood was used. Platelets were isolated using standard techniques. 25 MBq of ^{32}P was immersed in modified Ringer solution (ACO, Läkemedel) (7 ml Ringer sol. + sodium citrate 60 mg + glucos.ad.inf. 50 mg + aq. ster. ad. 10 ml). Platelet-rich plasma (PRP) was incubated with the isotope for 2.5 hours at 37°C , the solution being rocked slowly.

Registration of radioactivity:

Two GM tubes (Philips type 18507) were used to register the beta-radiation from the ^{32}P labelled platelets. The GM tubes were screened using rectangular blocks of lead with a wall

thickness of minimum 6 mm. The windows of the GM tubes were positioned immediately over the exposed ear arteries. The tissue below the vessel was removed and the vessel placed in a slit in a rectangular lead plate 6 mm thick (Fig. 1, paper III). The signals from the GM tubes were shaped using locally-built discriminators. The pulses were counted in scalers (Selectronix Scaler-Timer Model 47-21) and connected to a rate-meter (Selectronix Analyzer Model 45-22). The rate-meter output was continuously monitored on a pen recorder. The number of pulses per 100 seconds was registered at regular intervals using the scaler.

Perfusion fixation:

A small catheter (PA 50-60) was placed in the central artery of the ear 2-3 cm proximal to the anastomosis. Small amounts of Ringer lactate (Ca^{++} 1.8 mmol, K^+ 4.0 mmol, Na^+ 151.6 mmol, Cl^- 132.5 mmol and lactate 26.7 mmol/l) were used to prevent clotting in the catheter. Otherwise, perfusion of the vessel was avoided. All vessels were then perfused for 15 min at a constant pressure of 120 mm Hg with a specially-prepared fixative (composition: 0.2 M phosphate buffer 400 ml + 50 % glutaraldehyde 50 ml + 20 % dextran T 70 95 ml + dist. H_2O 455 ml). This fixative was used in all specimens both for light microscopy and SEM. Following fixation, the vessels were easily separated from their surrounding tissues using microsurgical techniques. Samples for SEM were divided lengthwise using microscissors. Specimens intended for light microscopy were mounted in double Acland microvascular clamps and kept in the same fixative as above for another 12 hours. The samples were then washed 4-5 times in Millonig's phosphate buffer and kept immersed in this buffer until processing for light microscopy or SEM began.

Histological processing:

The samples, still mounted in double vascular clamps, were dehydrated and embedded in paraffin. Longitudinal 4 μ thick step sections were cut and stained with Weigert-van Gieson stains. A few specimens were stained with phosphotungstic acid hematoxylin for fibrin and Giemsa and Gordon and Sweet's reticulin method. The findings were recorded using microphotography.

Processing for SEM:

The samples were dehydrated in increasing concentrations of alcohol and immersed in freon. At the critical point, the specimens were dried with carbon dioxide. The vessel segments were then mounted on metal discs with the intimal side up and coated with gold - palladium (200 Å thick).

SEM:

The specimens were examined in a Cambridge "Stereoscan" Mark II A or in a Zeiss Nanolab 2000 and photographed at standardized magnifications.

Registration:

Blood flow values (paper I) were recorded using a potentiometer recorder (Servogor RE 511, Kompensationsschreiber, Goerz Electro).

Radioactivity values (paper III) were recorded using a potentiometer recorder (Omniscribe recorder, model B 5217-5, Houston Instrument).

Statistical methods:

Standard procedures were used. The significance of differences between observations was calculated with Student's t test and Fisher's exact test.

REVIEW OF PRESENT INVESTIGATIONS

Blood flow in small arteries after end-to-end and end-in-end anastomoses: An experimental quantitative comparison (I)

Material and experimental procedure

Thirty rabbits, males and females, weighing between 3.0-5.0 kg, were used. Fifteen ETE and 13 EIE microvascular anastomoses were performed on the central arteries of the ear (diameters 0.8-1.2 mm). In 5 rabbits end-to-end anastomosis was performed in one ear and end-in-end anastomosis in the other. One group of 7 rabbits served as control. These rabbits underwent the same operative procedures except for transection and reanastomosis of the arteries.

The operative procedure, anastomotic technique, wound irrigation, temperature regulation of the rabbit and EMF have previously been described.

After preparing the arteries, blood flow was measured with EMF for 1-2 hours until a steady state was reached. The arteries were then placed in double microvascular clamps, transected and anastomosis performed according to the conventional ETE technique or using the EIE technique ad modum Lauritzen. After the release of the vascular clamps and cessation of anastomotic bleeding, blood volume flow was continuously recorded for 4 hours, flow values being registered every half hour. Blood flow recordings were also performed postoperatively on days 1 and 3. These flow recordings were continued until a steady state prevailed and this flow value was then registered. In the control group, blood volume flow was continuously recorded for 6 hours following preparation of the arteries. Flow values were registered every half hour as in the other groups. The procedures on days 1 and 3 were identical to those of the other groups. All flow values were calculated as percentages of the steady state rates obtained during the continuous recording 1-2 hours following preparation of the arteries.

Results (Fig. 2)

Thrombosis occurred in one anastomosis of each type.

In the control group, a slight increase in the mean blood flow was noted 3-4 hours after preparation of the vessels. The blood volume flow following the ETE technique did not change significantly from preoperative levels. Using the EIE anastomotic technique, blood volume flow decreased significantly ($p < 0.001$) throughout the entire period studied. The most serious reduction in blood volume flow was registered during the first 2 hours after release of the vascular clamps. The mean flow rate during the entire 3-day postoperative period was 93% using the ETE technique and 47% using the EIE technique. The difference between these values is highly significant ($p < 0.001$).

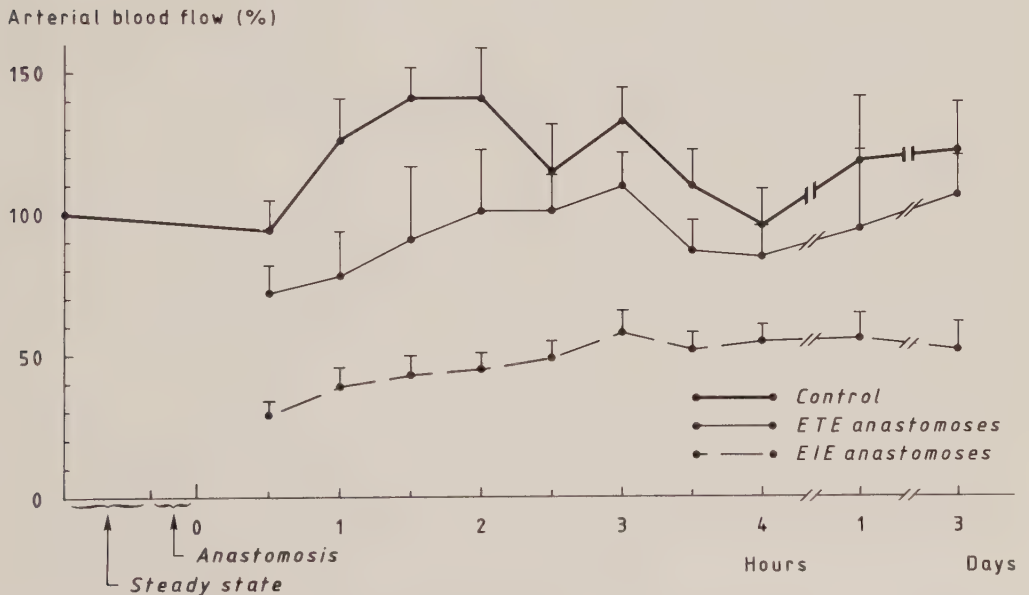


Fig. 2. Blood volume flow in the central arteries of rabbit ears registered by EMF. ETE and EIE anastomoses compared to the preoperative value (100%). Standard error of mean is indicated.

Using the ETE technique in one ear and the EIE technique in the other followed by simultaneous flowmetry, the difference in blood volume flow was confirmed.

In 5 of 14 ETE anastomoses, postoperative hyperemia was observed. Hyperemia was never found with the EIE technique.

Comments

To obtain comparable blood volume flow recordings in vessels, studies must be performed under standardized conditions. Great care was taken to keep the temperature constant and to irrigate the wound properly. To ensure good blood volume flow with low peripheral resistance in the ears, all rabbits were kept hyperthermic at $39.8 \pm 0.2^{\circ}$ C using a thermostatically regulated lamp and heating pad.

EMF values must be recorded carefully. The diameter of the flow probes must be chosen with care and it is advisable to use the same probe on any one vessel throughout the studies. Calibration of the equipment should be performed in vivo and repeatedly during the experiment (14,146,167,197).

The hyperemic reaction observed in the control group could be the delayed consequence of vasospasm during preparation of the vessels. It could also be due to an inflammatory reaction from release of local vasoactive metabolites. Hyperemic reaction was observed in the ETE group, but never in the EIE group. Using vessel ends of equal diameters, the luminal narrowing caused by the sleeve technique is severe and constitutes a critical "stenosis" when experimental conditions are as described above. Thus, even when peripheral resistance is reduced due to vasodilatation, which normally results in an increased blood flow, this has no effect on blood volume flow, since the luminal narrowing at the anastomotic site constitutes the main resistance to blood flow.

Stenosis following end-In-end microarterial anastomosis: An angiographic comparison with the end-to-end technique (II)

Material and experimental procedure

Thirty-five rabbits, males and females, weighing between 2.5-5.0 kg were used. Thirty-three ETE anastomoses were performed on the saphenous arteries or the central artery of the ear (diameters 0.8-1.2 mm). Thirty-six EIE anastomoses ad modum Lauritzen were performed on the same two types of arteries and also on 5 femoral arteries (diameters 1.7-2.0 mm) and 5 renal arteries (diameters 2.0-2.7 mm). Five EIE anastomoses ad modum Meier were performed on the central artery of the ear (diameters 0.8-1.2 mm).

The operative procedure, anastomotic techniques, measurements of vessel diameters and projection angiography were performed as described previously.

Angiography was performed on groups consisting of 5 ETE or 5 EIE anastomoses at intervals of one hour, 3, 7, 14, 30 and 90 days. Angiograms of femoral and renal arteries - 5 EIE anastomoses ad modum Lauritzen in each group - were taken only at one hour postoperatively. Using the EIE technique ad modum Meier on the central artery of the ear and on saphenous arteries, angiograms were taken only after an interval of one hour.

The luminal areas of the anastomoses were calculated according to the formula described previously.

Results

Occlusion was found in 3 ETE and 6 EIE anastomoses performed in small arteries (0.8-1.2 mm). They occurred immediately postoperatively or within two weeks. These vessels were excluded from the series.

ETE anastomoses in the central artery of the ear and in saphenous arteries (diameters 0.8-1.2 mm) (Fig. 3): One hour postoperatively, the mean luminal area of the anastomosis had decreased, the decrease being statistically significant ($p < 0.001$). In the other 5 groups a slight luminal narrowing was sometimes

observed between 3 and 90 days, but the decrease in luminal area was not statistically significant.

EIE anastomoses ad modum Lauritzen in the central artery of the ear and in saphenous arteries (diameters 0.8-1.2 mm) (Fig. 3): A severe luminal reduction was found at all intervals. The mean luminal area one hour postoperatively was only 22% of the vessel luminal area. The anastomotic diameters increased slowly during the following weeks and months. The mean luminal area at 7 days was 35% of its initial value and at 90 days 63%. The differences in luminal area between either normal vessels or the ETE group on the one hand and the EIE group on the other were significant ($p < 0.01$).

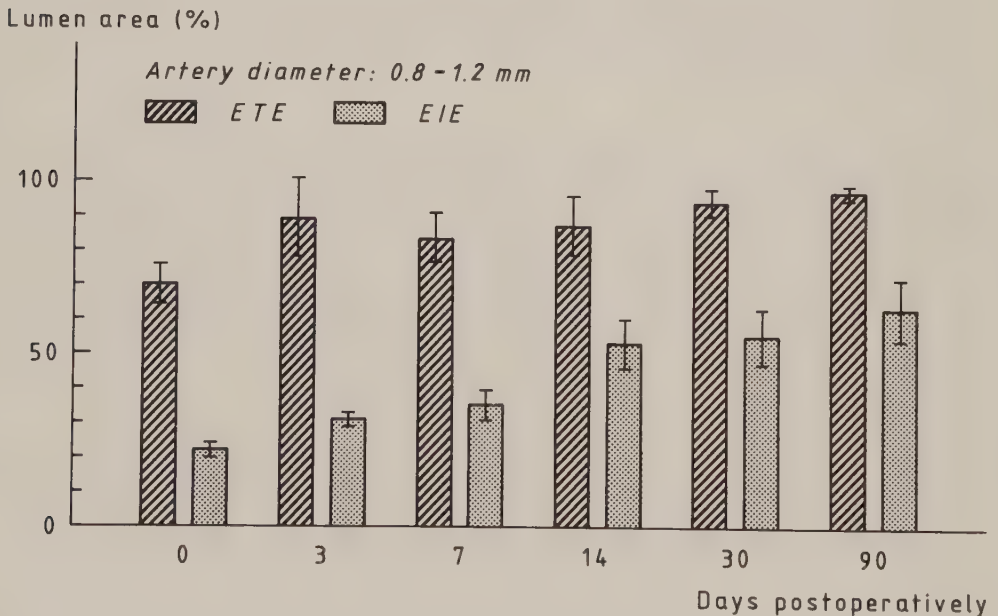


Fig. 3. ETE and EIE anastomoses in small arteries (diameter 0.8-1.2 mm). Each column represents mean luminal area of 5 anastomoses. Standard error of mean is indicated.

EIE anastomoses ad modum Lauritzen in femoral arteries (diameters 1.7-2.0 mm) and renal arteries (diameters 2.0-2.7 mm) (Fig. 4): The mean luminal area one hour postoperatively was 35% in femoral arteries and 48% in renal arteries.

EIE anastomoses ad modum Meier in central arteries of the ear and saphenous arteries (diameters 0.8-1.2 mm) (Fig. 4): The mean luminal area was one hour postoperatively 39% of the vessel luminal area. The difference in luminal area between the two EIE techniques (35% versus 22% by Lauritzen's technique) was statistically significant ($p < 0.01$).

Aneurysms were never observed using any of the techniques described.

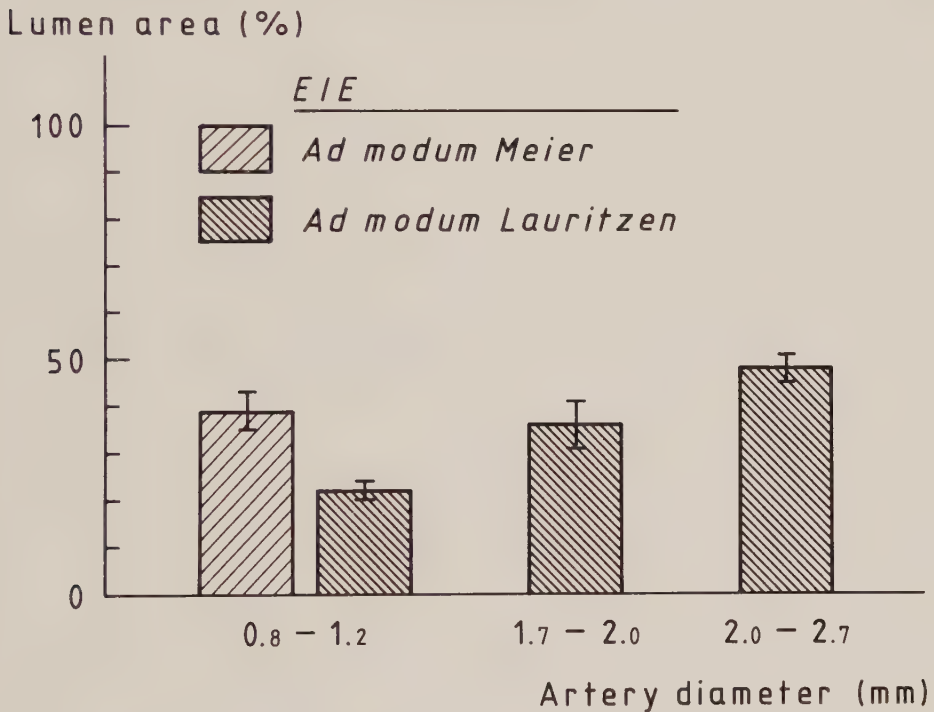


Fig. 4. EIE anastomosis ad modum Lauritzen in arteries of 0.8-1.2 mm, 1.7-2.0 mm and 2.0-2.7 mm in diameter. Each column represents mean remaining luminal area of 5 anastomoses. The mean luminal area in arteries of 0.8-1.2 mm in diameter is compared to 5 EIE anastomoses ad modum Meier. Standard error of mean is indicated.

Comments

The results are discussed in paper II and in the General Discussion.

Accumulation of isotope labelled platelets in small arteries after end-to-end and end-in-end anastomoses in the rabbit (III)

Material and experimental procedure

Eight rabbits, males and females, weighing between 3.0-4.0 kg were used. Six ETE anastomoses and 5 EIE anastomoses were performed on the central arteries of the ear (vessel diameters 0.8-1.2 mm). In 3 rabbits, an ETE anastomosis was performed on one ear and an EIE anastomosis on the other allowing simultaneous studies in the same animal.

The operative procedure, anastomotic techniques, labelling of platelets with ^{32}P and the registration of radioactivity have been presented previously.

The stability of platelets labelled with ^{32}P in orthophosphate was studied in blood samples from another 8 rabbits at intervals of 0.5, 1, 2, 3, 4, 5 and 6 hours after infusion of the labelled platelets. The activity of ^{32}P was measured at these intervals in the platelet fraction (PRP), plasma (PPP) and the remaining corpuscular fractions. All values of radioactivity were calculated as percentages of the value registered 0.5 hours after infusion of the labelled platelets.

The radioactivity in prepared arteries was recorded for 2 hours following infusion of ^{32}P -labelled platelets. The radioactivity levels reached a steady state within the first hour. Application of microvascular clamps, transection and anastomosis were then performed. Monitoring of the levels of radioactivity was continued for 2-4 hours following performance of the anastomosis, release of vascular clamps and cessation of bleeding at the anastomotic site. To avoid contamination with radioactive blood plastic films were placed around the artery and exchanged one or more times before starting to record activity.

Controls: In one rabbit, 25 MBq ^{32}P isotope in orthophosphate were injected intravenously instead of labelled platelets. The activity levels were followed over two central ear arteries

prepared and anastomosed as all other vessels.

In two prepared but not transected and anastomosed arteries, labelled platelets were infused and activity levels followed as in the other groups.

Bleeding was controlled repeatedly during each experiment. All vessels were inspected and checked for patency at the end of measurements. The radioactivity of the vessel segment was measured after excision and reapplication of the GM tube. Background activity was recorded at the end of all experiments after removing the vessel segment.

Results

Platelet labelling: The radioactivity in the platelet fraction was $65 \pm 3\%$ of the total blood activity 0.5 hours after the infusion of labelled platelets. The residual activity was mainly in the corpuscular fraction. The values of activity in the plasma fraction were low (1-4%). The activity in the platelet fraction during the interval 2-6 hours following infusion - the period of registration over the anastomoses - decreased from 61 to 39%. Whole-blood activity was also observed to decrease by about the same amount as the platelet fraction.

Controls: Following infusion of ^{32}P as the orthophosphate, no increase in activity was observed following ETE anastomosis.

Activity levels of prepared but not transected and anastomosed arteries remained at steady levels following the infusion of labelled platelets.

The vessel segment radioactivity at the end of the experiments corresponded to 85-95% of the registered activity.

A small increase in background activity was also noted at this time.

ETE anastomoses (Fig. 5): Activity at the anastomotic sites increased rapidly following removal of the vascular clamps. Maximum values ($414 \pm 94\%$) were obtained within 30 minutes. The radioactivity then decreased gradually during the next 2-4 hours. In one ETE anastomosis, a second peak was observed 2-3 hours after anastomosis, followed by a rapid decrease in activity. Patency was always checked at the end of measurements and was good in all ETE vessels, except in one vessel with thrombotic occlusion and high radioactivity values throughout the period of registration.

EIE anastomoses ad modum Lauritzen (Fig. 5): In the four patent vessels, the labelled platelet activity was only modestly increased, if at all. In one vessel occluded by thrombus (as confirmed by a patency test), the radioactivity increased as in ETE anastomoses and then remained high.

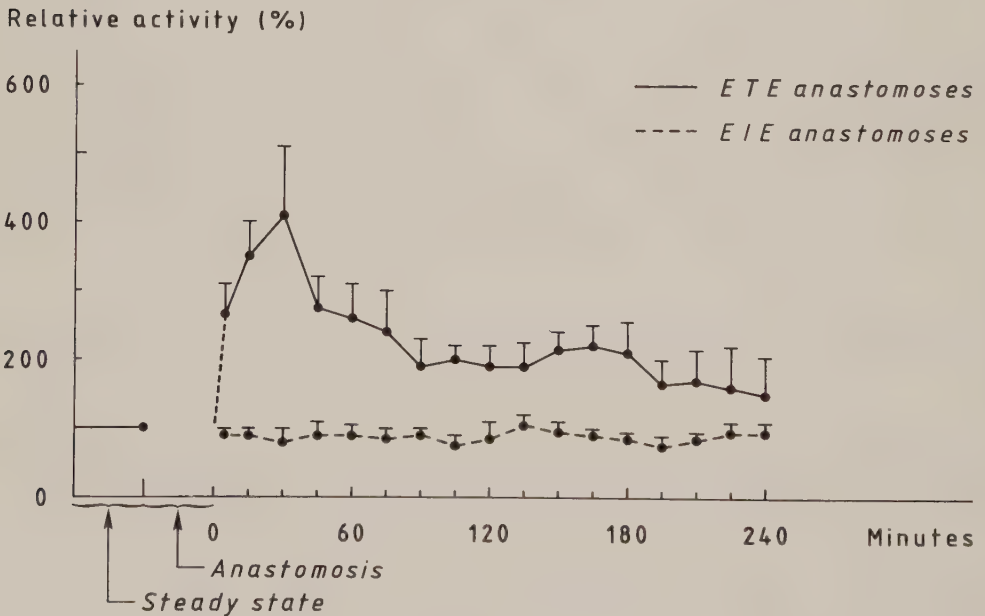


Fig. 5. Activities of ^{32}P labelled platelets following ETE and EIE anastomotic techniques. Standard error of mean is indicated.

Comments

The radioactivity in PRP samples 0.5 hours post infusion was 65% of the total blood activity, the residual activity being mainly in the blood corpuscular fraction. The reason for this is incomplete separation of platelets from the corpuscular fraction during centrifugation. The ratio of activity in the platelet fraction to that in the corpuscular fraction was essentially constant throughout the period, implying that there was no redistribution between different blood fractions. The net decrease in platelet radioactivity observed was probably due to release of ^{32}P and excretion or transfer to other body compartments.

The radioactivity did not increase significantly when using the EIE technique. This is surprising since platelet thrombi have been observed at the ends of telescoped segments (SEM and light microscopy). The lack of platelet accumulation could be due to the fact that the platelet aggregates were very small and to luminal narrowing following the sleeve technique implying a considerable decrease in the number of platelets in the vessel. A limited decrease in radioactivity in the platelet population during the registration period might be another factor which could conceal the expected increase in radioactivity. Observations in longitudinal specimens using light microscopy and SEM support the finding of smaller platelet aggregates following the sleeve technique as compared to conventional ETE techniques. The consistent high increase following the ETE technique depends on the large number of platelets required for sealing anastomotic gaps and suture holes and probably also on the thrombogenicity of the suture material.

A histological comparison of experimental microarterial end-in-end and end-to-end anastomoses (IV)

Material and experimental procedure

Rabbits, males and females, weighing between 2.5-4.0 kg were used. Twenty-five EIE and 21 ETE anastomoses were performed on the central artery of the ear (vessel diameters 0.8-1.2 mm). EIE anastomoses were performed ad modum Lauritzen.

The operative procedures, anastomotic techniques, fixation and processing of vessel specimens were performed as previously presented.

The profiles of the longitudinal vessel sections were studied using light microscopy. Groups of 3 vessels were studied at intervals of 1-2 hours and 1, 3, 7, 14, 30, and 90 days postoperatively. All specimens were stained with Weigert-van Gieson stains except 4 EIE specimens stained with phosphotungstic acid hematoxyline for fibrin and Gimsa and Gordon and Sweet's reticuline method. These 4 specimens were studied at day 7.

The vessel samples were documented by photography.

Results (see figures paper IV)

Thrombosis occurred in 2 ETE and 2 EIE anastomoses, in both instances, at 1-2 hours and at 3 days postoperatively.

At 1-2 hours: Severe luminal narrowing was observed in the telescoped segments of all EIE anastomoses. Platelet aggregates adhered to the ends of the telescoped segments. Pockets were frequently observed between the internal and external sleeves.

No obvious luminal narrowing was observed in ETE anastomoses. The defects between the transected vessel ends and around the suture holes were filled with platelets. Sometimes large aggregates of platelets mixed with fibrin and erythrocytes were formed.

At day 1, the findings were similar to those observed at 2 hours with the addition of atrophy and disintegration of the medial layers of the vascular walls. Leucocytes were found infiltrating the tissues around sutures, distally in the sleeved segment and also at the transected vessel ends following the ETE technique.

At day 3: The marked luminal narrowing following the telescope technique was beginning to recede. Infiltration of cells was observed both in the distal parts of the sleeved vessels and in platelet aggregates located nearby. The step between the internal and external sleeves was less prominent. Similar infiltration of cells was also observed following the ETE technique in vessel ends and platelet aggregates. Endothelial cells were sometimes observed on top of platelet accumulations.

At day 7: Platelet aggregates at the sleeved vessel ends were infiltrated with fibroblasts and loose connective tissue partially transformed the aggregates. Using special staining techniques, small islands of fibrin were observed in all platelet aggregates. The formation of loose connective tissue and invasion of fibroblasts were found in all medial layers using both EIE and ETE techniques. Capillary proliferation and polymorphonuclear leucocytes were observed in telescoped vessel ends. Endothelialization on top of transformed platelet aggregates and also in pockets between the double vascular walls was frequently observed. Organization of the platelet aggregates filling defects between transected vessel ends in ETE anastomoses was as described above. The formation of a hyperplastic neointima was sometimes observed following both techniques.

At day 14: Neo-endothelialization was almost complete following both types of anastomosis. All ETE vessels healed with widespread intimal hyperplasia. In EIE vessels intimal hyperplasia was mainly located distal to the sleeved vessel segment. Atrophy and thinning of the medial layers was always observed

following both ETE and EIE anastomosis. The luminal narrowing was now less obvious in EIE vessels, due to atrophy of the medial layers in both the external and internal sleeves described above.

At day 30: Following both anastomotic techniques, the intima showed a multilayered hyperplasia. The medial and adventitial layers following both techniques consisted mainly of heavy fibrosis.

At day 90: The observations were similar to those at 30 days. However, intimal hyperplasia seemed to be stagnating or even receding. The double vascular walls were still clearly visible following the EIE technique. Using light microscopy, neither stenosis nor aneurysm was observed with either technique.

Comments

The observations on longitudinal profile specimens using light microscopy explain the severe luminal narrowing following EIE techniques. The main reason is the doubling of the vascular walls. No compensatory dilatation of the outer sleeve was ever observed. On the other hand, pockets between the double walls, which might further reduce the inner luminal area, were frequently seen. Using the EIE technique according to Lauritzen's description, the inner sleeve is tucked into the distal lumen, without longitudinal tension in the telescoped segment, resulting in contraction and sleeve thickening, this being most pronounced towards the end. The internal elastic laminae in the sleeved segment were folded transversally as a result of such a contraction. Nothing similar was ever observed in other anastomoses. Platelet accumulation following the EIE technique was always so limited that it cannot account for the early luminal narrowing. The gradually increasing anastomotic luminal area following the EIE technique is mainly due to atrophy of the medial layers in both the external and the internal sleeves.

Endothelialization following end-to-end and end-in-end micro - arterial anastomoses. A scanning electron microscopical study (V)

Material and experimental procedure

Twenty-two rabbits, males and females, weighing 2.5-4.0 kg were used. Eighteen ETE and 18 EIE anastomoses were performed on the central artery of the ear (vessel diameters 0.8-1.2 mm). All EIE anastomoses were performed ad modum Lauritzen. Eight central arteries were used as controls.

The operative procedures, anastomotic techniques, fixation and processing of vessel specimens were performed as previously presented. The intima of the vessel segments was studied over the whole length of the specimens using SEM at standardized magnifications. Groups of 3 vessels were studied at intervals of 1-2 hours and 3, 7, 14, 30 and 90 days postoperatively. Control specimens were studied at intervals of 15 minutes and 2 hours postoperatively.

Eight vessel samples - 3 ETE anastomoses at 7, 14 and 90 days and 5 EIE anastomoses at 30 and 90 days - were studied using both SEM and light microscopy.

The vessel specimens were documented by photography at standardized magnifications.

Results

Controls: Normal endothelium consists of spindle shaped cells with smooth surfaces and with the long cell-axis oriented in the direction of blood flow. Villous projections are only seen at cell borders.

The repair process

Thrombotic occlusions were observed in one ETE vessel and one EIE vessel at 1-2 hours and at 7 days.

At 1-2 hours: Platelet aggregates, fibrin and erythrocytes were found between transected vessel ends, at sutures and suture holes and always at the cut end of the sleeved vessels. Severe endothelial damage with denudation was observed at anastomotic sites and also at clamp sites following both anastomotic techniques. Severe luminal narrowing and pockets between the internal and external sleeves were observed using the EIE technique.

At day 3: The border between exposed subendothelial layers and undamaged endothelium was distinct. The subendothelium was covered with flattened and extended platelets forming a temporary intima. Neo-endothelial cells were observed in damaged areas. Large numbers of leucocytes were found, mainly at anastomotic sites. The sutures were partly covered by platelets, fibrin and white cells. A thrombus always protruded from the telescoped vessel end.

At day 7: Reendothelialization occurred in all denuded areas. Using the EIE technique, neo-endothelialization was observed on top of the thrombus at the end of the sleeved vessel. Neo-endothelialization was observed by endothelial ingrowth from undamaged borders, but isolated endothelial cells were also seen in damaged areas. The neo-endothelium consisted of pleomorphic cells separated by deep clefts and holes.

At day 14: The endothelial sheet was almost completed. However, small defects and intramural thrombi were sometimes observed.

At day 30: The most conspicuous finding was an abnormal neo-endothelium with aberrant endothelial cells. Large defects and thick intercellular bridges were seen at cell junctions.

At day 90: The intima, following both anastomotic techniques, resembled those at 30 days.

Comparison between SEM and light microscopy observations

In 7 specimens (3 ETE anastomoses at 7, 14 and 90 days and 4 EIE anastomoses at 30 and 90 days), abnormal endothelium covered areas of intimal hyperplasia. At 90 days, the remaining EIE vessel showed evident intimal hyperplasia but an uneven endothelial sheet in which the individual cells looked almost normal.

Comments

The processing of specimens for SEM and the appearance of vascular endothelium are still matters of controversy. The selection of the solutions used prior to fixation seems to be crucial (90, 141, 184). The method of perfusion fixation under physiological pressure prior to preparation of vessel specimens in our opinion causes few artifacts.

The vessel samples taken at 1-2 hours show the intimal surface with platelet adhesion, aggregation and desaggregation. This initial phase following arterial damage is also studied in paper III. The severe luminal narrowing after EIE anastomosis is difficult to evaluate in SEM specimens. The "stenotic" effect of telescoping vessel ends is better judged from angiographic findings (paper II).

The time required for neo-endothelialization was similar using the two anastomotic techniques. Endothelialization following the EIE technique took place on top of the platelet thrombus, which was simultaneously transformed (paper IV), at the sleeved vessel end. The finding of single neo-endothelial cells in denuded areas and per continuitatem growth from undamaged endothelium favours both a blood-borne genesis of endothelial cells (71) and local multiplication of undamaged cells (66,169,165,185).

Observations using SEM and light microscopy suggest a strong connection between intimal hyperplasia and the development of an abnormal endothelium. The relationship between these abnormal cells and the deeper hyperplastic cellular layers is unknown.

GENERAL DISCUSSION

The failure rates in microvascular surgery are still higher than in vascular surgery on larger vessels. The problem of vascular occlusion is mainly related to the narrow lumen and low blood flow states, particularly in veins. Endothelial damage, e.g., from anastomotic procedures and clamping, is always followed by platelet thrombus formation (5,6,13,20,21,195). This process seals defects in the vessel and forms a temporary intima. However, in small vessels, this platelet thrombus formation might endanger the vessel patency. A narrow vessel lumen, large endothelial defects, exposed media and adventitia and decreased blood flow (190) frequently exaggerate thrombus formation, which might lead to anastomotic failure. Improved microscopes, instruments and sutures have made high patency rates in experimental microsurgery possible even in vessels down to 1 mm in external diameter. However, in clinical microsurgery patency rates have been lower. Different anastomotic techniques are available and a number of antithrombotic regimens are used to counteract anastomotic occlusion (56). New in vivo methods must be developed in order to study the pathophysiological events behind vascular occlusions. Such methods will enable the correct evaluation of different microvascular procedures and the use of pharmacological agents.

To understand the mechanisms of vascular occlusion, it is necessary to study functional and morphological aspects of microvascular anastomosis. The animal model used in these experiments was specially designed to study the effect of microvascular anastomoses on blood volume flow and platelet accumulation. A prerequisite for obtaining reliable results was a raised body temperature of the rabbits leading to peripheral vasodilatation and high and steady flow levels. The experimental model allowed studies on early and late events of thrombus formation by an in vivo tracer technique, light microscopy (profile) and SEM (intima) of vessel samples. Until the anastomotic site is healed, the risk for thrombus formation remains and in addition to blood flow and thrombus formation the repair processes and remodelling of the anastomosis are the main aspects to consider in

studies of anastomotic techniques. Blood flow in tissues can be estimated in a number of ways (102,103,159), but only few methods are available for registering blood volume flow in vessels in vivo (12,93). Electromagnetic flowmetry was previously used for large vessels and is regarded as a most accurate method (14,52,75,93,146,160,167,197). EMF probes intended for use on small vessels (diameters about 1 mm) have recently become available commercially and the present study used EMF on such small arteries for comparison of two anastomotic techniques. The limitations in using EMF do not depend on the technical device itself (14) but meticulous attention to the experimental setup (e.g. environmental and animal temperature, anesthesia, wound irrigation) and repeated calibrations of the instrument in vitro and in situ are necessary.

Thrombus formation in small arteries is mainly due to accumulation of platelets. Low flow states, as in veins, increase the proportion of fibrin to platelets in thrombus formation following vascular trauma (154). Platelet accumulation was thus followed in small arteries using as platelet label a beta-emitter (^{32}P), making these platelet accumulation studies possible. The difficulties of isolating the activity emanating from the short, narrow vessel segment studied from that of the background is well-known using ^{51}Cr (1) or ^{111}In (72).

Furthermore, using ^{111}In , vessels have to be photographed with a gamma-camera at intervals and the important information available from continuous in vivo registration is then lost.

The two techniques for microvascular anastomosis that were studied and compared were the conventional ETE technique and the recently introduced EIE technique.

The principles behind the ETE anastomosis have been developed so as to reduce the risk of thrombus formation (6). Beside gentle handling of the tissue, these include limited stripping of superfluous adventitia at vessel ends and correct placing of single sutures (10,32,88,150). Earlier recommendations by some authors to place single sutures only through the adventitial and medial vascular layers thus preventing the exposure of foreign

material intraluminally have been abandoned because the intima was found to retract at the anastomotic line leaving large sub-endothelial defects. The use of a continuous suture may cause stenosis at the anastomotic site. However, recently Hamilton et al. (1979) (81) using double clamps and stay sutures found equal patency rates in small vessels in a comparison of single and continuous suture methods. The handling of continuous sutures is, however, difficult in the limited field of vision of a microscope. Placing holding and interrupted sutures and turning the vessel have all been designed to avoid trapping the rear wall (50,51) and permit inspection of the lumen before final closure of the vessel. Operative techniques i.e., oblique cuts of vessel ends, fish-mouth cuts and the use of a vein patch (57) at the anastomotic site were introduced to minimize luminal narrowing, but are mainly used when there is a discrepancy in vessel diameters (53,84).

In 1978, two different EIE techniques were introduced by Lauritzen (115) and Meier (127) as time-saving and simple techniques with patency rates similar to those obtained using ETE techniques. One of the objects was to eliminate any suture material intraluminally by bites of the adventitia and media of the vessel wall (Fig. 1). However, fundamental microvascular principles were violated. Doubling the vascular walls must presumably cause some luminal narrowing and at the same time one vessel end is left exposed in the vessel. Severance of an artery leads to longitudinal contraction. Using the telescope technique proposed by Meier, this problem is counteracted by pulling the sleeved segment into the distal vessel lumen. Using that advocated by Lauritzen, the sleeved segment is tucked into the distal vessel and consequently some unfavourable contractions may occur in the sleeved vessel segment. This also seems to be the result as shown in paper IV.

Telescope techniques are quicker and easier to perform than conventional ETE techniques, this being particularly true in small vessels. Using larger vessels, several additional sutures are usually needed to stop bleeding. In constructing sleeve anastomoses ad modum Meier, a major problem is that the sutures must

be placed symmetrically, since otherwise serious distortion of the vessel occurs. Another advantage of the microvascular sleeve technique is that the risk for intimal dissection that may occur with the ETE technique is minimized. However, even in ETE anastomosis, intimal dissection is rarely observed when the sutures are placed with full thickness bites through the vessel wall. Still another advantage of the telescope technique is that so far aneurysms have not been reported (118,123,126).

Patency rates using telescope techniques have been reported to be equal to or only slightly less than those found using conventional ETE techniques (48,111,117,143,174,189). We report a modest increase in the number of thrombi when the sleeve technique is used. The total number of occluding thrombi in the ETE group was 9/103 and in the EIE group 12/93.

The luminal narrowing in the two types of anastomoses studied differs considerably. A significant luminal narrowing was observed in ETE anastomoses only at 2 hours. Visual inspection postoperatively revealed local vessel dilatation both at anastomotic sites and at clamp sites, separated by segments of vessels which looked constricted. These findings were confirmed in angiograms of the vessels. In dilated areas, the intima studied with SEM was devoid of longitudinal endothelial folds. The reason for dilatation is the damage to the internal elastic lamina and the medial vascular layer, resulting in paralysis of the muscular media. Our finding of a small temporary decrease in luminal area could be the result of either vasospasm in prepared vessel segments, platelet thrombus formation or thickening of the vascular wall due to inflammation and oedema following the surgical procedure, or to any combination of these factors. When the sleeve techniques were introduced, the unavoidable luminal narrowing caused by doubling the vascular walls was regarded as a minor problem. However, in our angiographic study, the degree of luminal narrowing observed using arteries with vessel ends of equal diameters was found to be considerable, the reduction in luminal area being more than 50% during the first postoperative week. During the following weeks and months, the anastomotic luminal area increased. This was due to atrophy of the medial layers of

the double vascular walls. Such atrophy has been reported to be part of the normal process of vascular healing (107,108). When profile specimens were studied using light microscopy, the sleeved segment was found to be contracted in the longitudinal direction and frequently the internal sleeve did not adhere to the external sleeve. Pockets between the internal and external sleeves were observed in all vessels during the first week and in 50% up to 3 months postoperatively (111). That the contraction in the sleeved segment was counteracted by pulling the proximal vessel end into the distal vessel lumen with the sutures was confirmed in our angiographic studies. With the Meier technique luminal narrowing was somewhat less than using Lauritzen's technique.

The small reduction in blood volume flow in ETE anastomoses at 0.5 and 1 hour postoperatively, was probably depending on vasospasm in the prepared segment and/or on luminal reduction due to formation of platelet thrombus at the site of the anastomosis.

The corresponding reduction in blood volume flow using the EIE technique was about 50% and must be regarded as considerable (138). The immediate postoperative period is the most crucial as regards the risk of serious thrombus formation and limitations in blood volume flow may increase this risk. The reduction in blood volume flow following the sleeve technique is probably due to the severe luminal narrowing caused by the doubling of the vascular walls, since early thrombus formation was low (174). The intraluminal pressure in the narrow sleeved segment is decreased due to both increased flow velocities and turbulence both proximal and distal to the narrow segment. Thus, there is no reason for a dilatation of the external sleeve, as is often illustrated. In fact, the internal sleeve was observed to contract, resulting in the formation of pockets between the internal and external sleeves. The Meier technique, preserving longitudinal sleeve tension, probably is somewhat more favourable in this respect.

The sleeve technique using vessel ends of equal diameters resulted in axisymmetric biological stenosis (23). The diameter using the sleeve technique was halved 1-2 hours postoperatively. According to the Poiseuille equation* (158), this would lead to a reduction in blood volume flow to one-sixteenth of the pre-anastomotic steady-state value. Our flow studies only gave a volume flow reduction of about 50%. This illustrates the fallacy of applying the Poiseuille equation to blood flow through vessels with local narrowing, since it was originally derived from experiments with Newtonian fluids in tubes of constant diameters (38,64,65). Therefore, in blood vessels and using non-Newtonian fluids, as shown experimentally, the four parameters: radius, pressure drop, length of stenosis and viscosity affect blood volume flow to a much smaller degree than predicted by the equation (80,121,124,168,170,178,180,196). A narrowed vessel segment giving a reduction in blood volume flow is called a "critical stenosis". The decrease in vessel radius causing "critical stenosis" is related not only to the appearance of the segmental narrowing, but also to blood flow velocity, blood pressure, turbulence and the prevailing peripheral resistance (38,64,65). Thus, basic physiological parameters such as temperature and the functional requirements of the target organ determine blood volume flow through a supplying artery at any specific time. Whether or not a stenosis is "critical" therefore depends on these parameters, which may change. In the standardized conditions of the present study, the reduction of blood volume flow caused by the EIE technique indicates critical stenosis. The special problems in microvascular surgery with hyperemic reactions due to previous ischemia (175) or inflammatory reactions, as well as the no-reflow phenomenon (82,105,125,186,202), are related to the target organ and hence will not be discussed here.

$$Q = \frac{\Delta P r^4 \pi}{8\eta L}$$

where Q = flow, P = differential pressure at the two ends of the tube, r = the radius of the tube, η = viscosity of the fluid, and L = length of the tube.

The first stages of vascular repair include platelet accumulation (13,20,21,154). The constant high accumulation of platelets following the ETE technique is due to defects at the anastomosis and the suture holes. Suture material is also known to be highly thrombogenic (112). Platelet accumulation reaches a maximum within 30 minutes and is followed by a slow decrease to a lower level confirming Acland's observations of platelet thrombus formation in small vessels (5,6,173).

That the initial formation of platelet thrombus is remarkably small following the EIE technique is confirmed both in histological specimens and by the low values of radioactivity measured, using labelled platelets. Besides the reduction in blood volume flow the severe luminal narrowing probably also gives turbulent flow. Such factors usually increase platelet thrombus formation. On the other hand, the high flow velocities caused by the narrow sleeved segment might counteract these tendencies and protect the EIE anastomosis from early failures. As to the influence of various antiaggregating factors, one can only speculate. Due to the intact endothelial sheet of the external sleeve, the production of the antiaggregatory prostacyclin PGI_2 might be more efficient than in ETE anastomosis. The production of the proaggregatory endoperoxydes, $\text{PGG}_2 - \text{PGH}_2$, in the sleeved segment, is probably of minor importance. Another factor that might contribute to protecting the sleeve anastomosis from failure could be a high production of plasminogen activators from the severed vessel end.

The first stages of vascular repair following microvascular anastomosis were studied using labelled platelets and the later histopathological events were studied and compared using light microscopy and SEM (18,83,140,169,185). Perfusion and fixation under physiological pressure using a specially designed fixative allowed simultaneous comparison of the endothelialization and repair processes of the vessel wall (184). The methods used have been developed to minimize unfavourable influences on the specimens during the preparation procedure (90). The results from control specimens indicate that the methods are appropriate. The time for neo-endothelialization in our study was the same for

both techniques. Three points of particular interest were observed.

- 1) Intramural thrombi, using both techniques, could be observed although the process of endothelialization seemed to be completed.
- 2) Abnormal endothelium was found at least three months postoperatively.
- 3) An abnormal endothelial sheet was strongly correlated with intimal hyperplasia for at least three months.

The results suggest that the process of reendothelialization can occur by cell division from blood-born cells and/or cells from deeper wall layers (66,71, paper V ref. 24).

In the present thesis techniques were developed enabling the study of blood volume flow and platelet thrombus formation after microvascular anastomosis. Using these methods and comparing two microarterial anastomotic techniques (ETE and EIE) has increased our knowledge of healing processes as well as the basis for complications in microarterial anastomosis. The EIE technique studied was disadvantageous regarding luminal narrowing and decrease of blood volume flow, but as an experimental model has contributed to the understanding of vascular response to trauma and the repair process.

The methods used are tools to analyze the mechanisms behind vascular occlusion. Furthermore, these techniques will be used in future examinations of the thrombogenicity of different suture materials, the action mechanisms and effectiveness of anti-thrombotic agents and to evaluate new microvascular techniques.

CONCLUSIONS

1. The methods developed for studying blood volume flow and platelet accumulation enable continuous in vivo registration of these parameters following trauma to small blood vessels, such as anastomotic procedures.
2. Blood volume flow following end-to-end anastomosis in small arteries (diameter 0.8-1.2 mm) was not significantly altered compared to pre-anastomotic flow values.
Blood volume flow following end-in-end anastomosis in small arteries was reduced by 50% up to 3 days postoperatively compared to pre-anastomotic values.
3. End-to-end anastomosis in small arteries caused a slight luminal narrowing one hour postoperatively but at 3, 7, 14, 30 and 90 days postoperatively no change in luminal area was observed.
Following end-in-end anastomosis ad m. Lauritzen in small arteries, a severe reduction in luminal area was observed up to one week postoperatively. A gradual increase in the remaining luminal area occurred up to 90 days postoperatively. Luminal narrowing following end-in-end anastomosis becomes less pronounced with increasing vessel diameters.
The immediate reduction in luminal area following end-in-end anastomosis ad m. Meier in small arteries is somewhat less severe than end-in-end ad m. Lauritzen.
4. The accumulation of platelets in patent microarterial end-to-end anastomoses is more pronounced than in end-in-end anastomoses.
5. Luminal narrowing using the end-in-end technique depends on the doubling of the vascular walls. The gradual increase in luminal area following use of the end-in-end technique is mainly due to atrophy of the medial layers in the double vascular walls.
Neointimal hyperplasia is a frequent finding inside fibrotic medial and adventitial layers following both end-in-end and end-to-end anastomosis.

6. Reendothelialization is completed between 7 and 14 days following both end-to-end and end-in-end microarterial anastomoses.
7. The endothelial sheet is frequently abnormal with irregular endothelial cells and defect cell borders in areas of intimal hyperplasia up to 3 months postoperatively.

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BLOOD FLOW IN SMALL ARTERIES AFTER END-TO-END
AND END-IN-END ANASTOMOSES

An experimental quantitative comparison

Jan B. Wieslander, MD and Magnus Åberg, MD

Microvascular anastomoses were performed under standardized conditions on the central artery of the ear in 23 rabbits. Fifteen end-to-end and 13 end-in-end anastomoses were compared. No anticoagulants or vasodilating agents, other than the local application of lidocaine, were used. Blood flow was measured by electromagnetic flowmetry before anastomosis and after anastomosis at intervals of 30 minutes for 4 hours and at 1 and 3 days. The validity of the electromagnetic flowmetry method was established in a control group of 7 rabbits. The mean volume of blood flow after end-to-end anastomosis was 93% of the preoperative value ($P > 0.05$). The corresponding mean volume of blood flow after end-in-end anastomosis was 47% of the preoperative value ($P < 0.001$). The difference in flow between end-to-end and end-in-end anastomosis was highly significant ($P < 0.001$). Microangiography demonstrated marked stenosis in the end-in-end anastomoses but not in the end-to-end anastomoses.

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BLOOD FLOW IN SMALL ARTERIES AFTER END-TO-END AND END-IN-END ANASTOMOSES: AN EXPERIMENTAL QUANTITATIVE COMPARISON

JAN B. WIESLANDER, MD, and
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Microvascular surgery was introduced 20 years ago,¹ and the number of clinical applications has expanded rapidly since that time² aided by great advances in technique and instrumentation.

In the past, the patency of an anastomosis has been judged by visual inspection and by a simple patency test.^{3,4} These evaluations, however, only give a rough estimate of the amount of blood flowing through an anastomosis. A quantitative method for measuring the flow of blood through an anastomosis is needed so that the various anastomotic techniques and the influence of drugs on blood flow can be evaluated objectively. Electromagnetic flowmetry (EMF) is regarded to be superior to other methods for measuring blood flow in small vessels.⁵ Recently,

electromagnetic flowmetry probes intended for use with vessels as small as 1 mm in diameter have become commercially available, making studies of changes in blood flow through microvascular anastomoses possible.

The end-to-end (ETE) microvascular anastomotic technique is well established.^{6,7} In 1978, the end-in-end (EIE) anastomotic technique (sleeve or telescoped anastomosis) was introduced as a method that is technically simpler than the ETE anastomosis in small-sized vessels.^{8,9} A quantitative comparison of these 2 anastomotic techniques has not as yet been reported.

The study reported here compares the volume of blood flow through ETE and EIE anastomoses as determined by electromagnetic flowmetry.

MATERIALS AND METHODS

Thirty male and female rabbits, weighing between 3.0 and 5.0 kg, were used in the study. The rabbits were kept on a standard pellet diet and were given water ad libitum.

All of the anastomoses were performed on the central artery of the ear. This artery is readily accessible and has a diameter of 0.8–1.2 mm. End-to-end anastomosis was performed in 10 rabbits; end-in-end anastomosis was performed

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Figure 1. A photograph of the experimental set-up. The rabbit's ear is fixed to a plastic mold with needles and the electromagnetic flow probe is fixed in the correct position on the vessel. The wound is irrigated with Tyrode solution and covered with a thin plastic film.

in 8 rabbits. In another 5 animals, ETE anastomosis was performed on the artery in 1 ear and EIE anastomosis was performed on the artery in the other ear of the same animal; blood flow measurements were recorded simultaneously. A group of 7 rabbits served as controls and underwent the same procedures as the study rabbits without division and anastomosis of the artery. The same surgeon performed all of the procedures (J.B.W.).

The temperature of each rabbit was maintained at $39.8^{\circ}\text{C} \pm 0.2^{\circ}$ during the entire procedure with a thermostatically regulated lamp. No anticoagulants or vasodilating agents were used other than the local application of lidocaine (Xylocaine, 10 mg/ml, AB Astra, Södertälje, Sweden). Tyrode solution without magnesium sulfate was used as an irrigant (Tyrode solution: 6.8 g sodium chloride, 0.4 g potassium chloride, 0.2 g calcium chloride with 6 parts water, 144 mg sodium diphosphate with 2 parts water, 2.2 g sodium bicarbonate, 1.0 glucose, and CO_2 -free sterile water to 1,000 ml). The magnesium sulfate was omitted because it has been shown to decrease platelet adhesiveness.¹⁰ Dermalon sutures (10-0, Davis & Geck) were used in all of the anastomoses.

Blood flow was measured with electromagnetic flow probes with openings of 1.0 mm and 1.2 mm (Nycotron AS, Oslo, Norway). Probe size was chosen to fit the vessel closely, and the probe was oriented to the axis of the vessel so that the magnetic field was perpendicular to flow. Care was taken to keep the probe electrodes clean, to remove blood from the vessel wall, and to avoid

kinking of the vessel. The environment was kept moist and the probe was fixed precisely. By taking these precautions and by calibrating the instrument *in vivo* before, during, and after each experiment, the accuracy is reported to be $\pm 2\%$ for flow rates down to 1 ml per minute.^{11,12} Flow rates were registered graphically and recorded as mean flow in milliliters per minute.

In addition to EMF, microangiography was performed with a primarily enlarged picture according to the method of projection micro-radiography.¹³ Urografin (Schering AG, Berlin, West Germany) was injected intra-arterially through a small catheter placed in a lateral artery in the ear. Fuji X-ray film (Fuji Photo Co., Ltd., Tokyo, Japan) was used.

All of the EMF values were evaluated statistically by analysis of variance, Student's *t* test, and Fisher's exact test.

Operative procedure. The rabbits were anesthetized with injections of sodium pentobarbital (Mebumal Vet., 60 mg/ml, Aco Laekemedel AB, Solna, Sweden); anesthesia was maintained with intermittent injections.

Proper flowmetry on small vessels requires precise fixation of the probe. Therefore, both the ear and the probe were secured with needles as a stable unit in a soft plastic mold, which was shaped in the form of a negative print of the rabbit's ear (Fig. 1). This arrangement allowed the ear to be moved without changing the probe/vessel relationship.

A 3×2 -cm flap was raised over the central vessels of the ear and the artery was exposed. Small amounts of lidocaine were used locally to counteract vasospasm. The wound was continuously irrigated with the modified Tyrode solution. The EMF probe was positioned over the exposed vessel and blood flow was measured for 1–2 hours until a steady state was achieved; this was regarded as the preoperative value (Fig. 2). After the preoperative value was obtained, the vessel was subjected to anastomosis by 1 of the 2 techniques.

ETE anastomosis. An Acland double microvascular clamp was placed on the artery, the flow probe was removed, and the artery was divided. Superfluous adventitia was excised for a distance of 1–2 mm at each end of the cut vessel. Three stay sutures were placed at 120° intervals and a minimal number (3–5) of interrupted sutures were placed between each of the stay sutures.

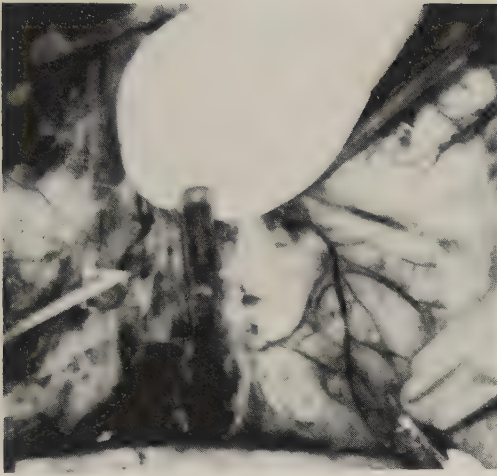


Figure 2. The electromagnetic flow probe in situ. The probe has been applied to the vessel so that the magnetic field is perpendicular to the direction of blood flow. Magnification: $\times 8$.

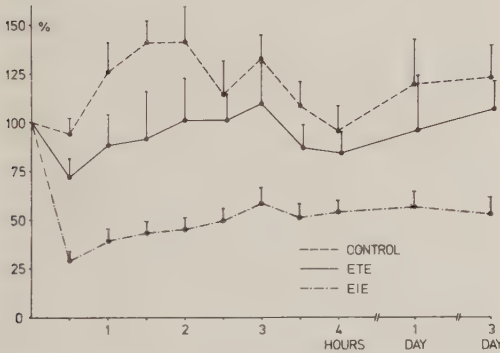


Figure 4. A graph depicting the percentage of blood flow in the arteries in the rabbit ears as registered by electromagnetic flowmetry after 14 end-to-end anastomoses (—) and 12 end-in-end anastomoses (---) compared to the preoperative value (100%). The dashed line (----) represents the values obtained in the 7 control rabbits. (Mean $\pm$ SE).

EIE anastomosis. The EIE anastomosis was performed according to the technique of Lauritzen (Fig. 3).⁸ The vessel was placed in a double adjustable clamp and divided. Loose adventitia was removed for a distance of approximately 2 mm from the end of the proximal stump and approximately 1 mm from the end of the distal stump. Two sutures were placed as follows: the first suture was begun by inserting the needle

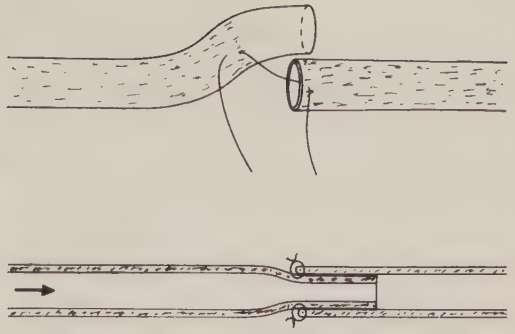


Figure 3. The construction of the end-in-end anastomosis according to the technique of Lauritzen.⁸ (Top): The placement of the initial suture is demonstrated in side view. (Bottom): The proximal vessel stump has been inserted (telescoped) into the lumen of the distal vessel stump in a cross-sectional view seen from above. The arrow indicates the direction of flow.

through the adventitia and part of the media 2 mm from the end of the proximal stump. The suture then was continued with a full-thickness bite through the wall of the distal stump close to its end. The second suture was placed in the same manner on the opposite side. The proximal stump then was inserted (telescoped) into the distal lumen. When necessary, a third and fourth suture was placed extraluminally midway between the first 2 sutures. Proper construction of this anastomosis keeps the lumen free of foreign material.

Following anastomosis, blood flow was recorded at intervals of 30 minutes for 4 hours. EMF was repeated 1 day and 3 days after surgery in the 10 rabbits with the ETE anastomoses and the 8 rabbits with EIE anastomoses. Values were recorded once a constant flow was established.

RESULTS

Blood flow. One animal in each of the ETE and EIE groups developed thrombosis and was excluded.

A slight increase of the mean blood flow was found in the control group, with maximum being observed at 1–2 hours ($P < 0.05$). The flow rates before performing the anastomoses ranged from 3–28 ml/min. Blood flow through the ETE anastomoses was not significantly altered from the preoperative levels. On the other hand, a highly significant decrease ($P < 0.001$) was found quantitatively in the blood flow through the EIE

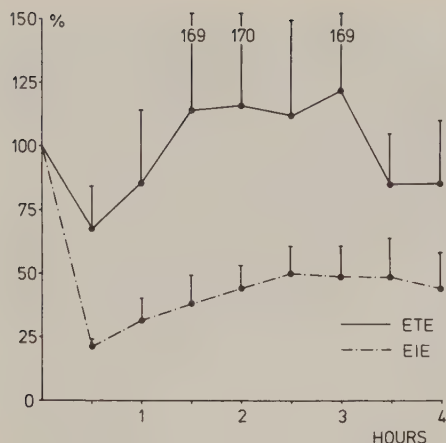


Figure 5. A graph depicting the percentage of blood flow in the arteries in the ears of 5 rabbits that underwent end-to-end anastomosis (—) in one ear and end-in-end anastomosis (---) in the other ear as registered by electromagnetic flowmetry and compared to the preoperative value (100%). The values were recorded simultaneously for each rabbit. (Mean $\pm$ SE).

anastomoses (Fig. 4) with the lowest rates occurring after 0.5–1.0 hour. The flow then slowly increased over 1–2 hours. While the mean volume of flow was 93% of the preoperative value in the ETE anastomoses 3 days postoperatively, it was only 47% of the preoperative value in the EIE anastomoses. The difference between these 2 values is highly significant ($P < 0.001$).

When flowmetry was performed simultaneously following ETE and EIE anastomoses in the same rabbit, the difference in the flow rates was confirmed (Fig. 5).

A postoperative increase in flow of 150%–300% was seen in 5(36%) of 14 ETE anastomoses, but was not observed in any of the EIE anastomoses ($P < 0.03$). This hyperemic reaction was delayed 1–2 hours, reached a maximum after 2–3 hours, and then slowly subsided. The initial postoperative flow pattern in the vessels with hyperemic reactions was similar to that of the majority of vessels.

Postoperative angiography showed a marked difference in luminal diameter between the ETE and the EIE anastomoses. Figure 6 shows a representative example of the radiographic findings on the first postoperative day. The average cross-sectional area of the lumen in the EIE anastomoses was only 16% of the cross-sectional area of the normal vessel lumen. The ETE anas-

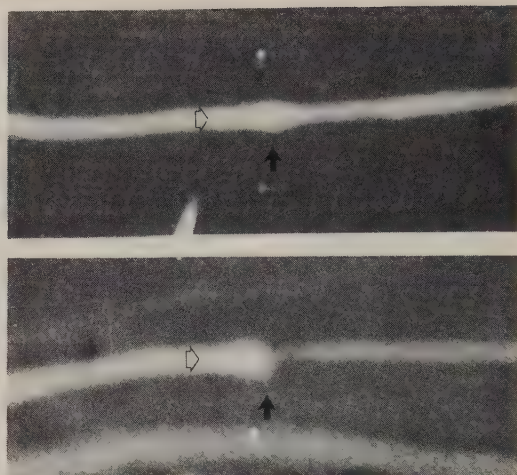


Figure 6. Microangiograms of the end-to-end anastomosis (top) and the end-in-end anastomosis (bottom) on the first postoperative day. The cross-sectional luminal area in the end-in-end anastomosis is decreased by 84%. The end-to-end anastomosis, on the other hand, shows slight dilation. Solid arrows point to the anastomosis; the open arrows indicate the direction of blood flow. Magnification: $\times 10$.

tomoses had no measurable effects on cross-sectional luminal area.

DISCUSSION

Because of the methodologic difficulties involved, little is known about blood flow through microvascular anastomoses in vivo.

The experimental model used in the present study seems to be valid. The preparation of the microvessel, including gentle removal of periadventitial tissue, ligation of side branches, and exposure, appears to have no adverse effect on blood flow. To provoke increased flow the animals were kept hyperthermic at $39.8^{\circ}\text{C} \pm 0.2^{\circ}$. Average flow rates were considerably lower after EIE anastomoses than after the conventional ETE technique. The number of thromboses was the same in both groups. The difference found in the blood flow rates agrees with the microangiographic findings. In the representative angiographic picture (see Fig. 6), the EIE anastomosis resulted in a considerable stenosis of the cross-sectional area of the lumen, which was decreased by 84%. The stenotic effect of the EIE anastomoses resulted in a mean reduction of flow to 47% of the preoperative rates. This reduction in flow was related to a decrease in luminal area and corresponds well with the decrease in

flow seen following experimental arterial stenosis, which has been reported by several authors.¹⁴⁻¹⁶

Our results clearly show that with regard to quantitatively higher blood flow, the ETE anastomosis is preferable to the EIE anastomosis in small-sized vessels (0.8–1.2 mm) during the first 3 postoperative days. In clinical practice this initial postoperative period seems to be the most critical one.¹⁷

Of note was the occurrence of 5 (36%) hyperemic reactions in vessels subjected to ETE anastomosis. No flow volumes above the preoperative level were seen in the EIE anastomoses. A comparison between ETE anastomoses and the control group revealed that the mean flow values after the ETE technique were lower, but otherwise followed the same course (see Fig. 4). It is interesting that the hyperemic reactions in the ETE group were often more pronounced, but otherwise coincided with the flow increase seen in the control group. The post-

operative hyperemic reaction is a well-known phenomenon in large vessel surgery.¹⁷ In animal experiments the magnitude and duration of hyperemic flow was shown to be proportional to the period of ischemia.¹⁸ Vascular spasm during preparation of the vessels and clamping during anastomosis causes tissue ischemia and may explain the hyperemic reactions seen in our study. The release of local vasoactive metabolites can also provoke hyperemia. The inability of a stenotic blood vessel to respond to a provoked hyperemic reaction is an indirect measure of the degree of stenosis,¹⁹ i.e., the stenosis becomes "critical" when the peripheral resistance is decreased.²⁰ Perhaps the hyperemia seen in our study can be regarded as a built-in test for vascular stenosis, since all vessels in the stenotic EIE group failed to show this reaction. The physiology of the hyperemia following microvascular surgery, as well as possible clinical applications of this phenomenon, is currently being studied in our laboratory.

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II

STENOSIS FOLLOWING END-IN-END MICROARTERIAL ANASTOMOSIS

An angiographic comparison with the end-to-end technique

Jan B. Wieslander, MD and Magnus Åberg, MD

Thirty end-to-end (ETE) and 30 end-in-end (EIE) microvascular anastomoses were performed in the central arteries of the ear or the saphenous arteries of 30 rabbits (diameters: 0.8–1.2 mm). The anastomoses were then examined angiographically at varying intervals postoperatively. The ETE anastomoses caused no stenosis in the majority of vessels, while the EIE anastomoses generally resulted in considerable stenosis. This was most marked one hour postoperatively, with the average luminal area in cross-section being 22% of the luminal area of the vessel. With time the stenosis in the EIE anastomoses gradually became less pronounced, but even after 90 days the cross-sectional luminal area was only 63% of the luminal area of the vessel. In a second series, EIE anastomoses were performed in five femoral arteries (diameter: 1.7–2.0 mm) and five renal arteries (diameter: 2.0–2.7 mm). The EIE technique was found to cause less pronounced stenosis in the larger vessels. In a third series, five EIE anastomoses were performed in the central arteries of the ear according to the technique of Lauritzen and compared with five EIE anastomoses performed according to the technique of Meier. The Meier technique was more difficult to perform but produced less stenosis.

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STENOSIS FOLLOWING END-IN-END MICROARTERIAL ANASTOMOSIS: AN ANGIOGRAPHIC COMPARISON WITH THE END-TO-END TECHNIQUE

JAN B. WIESLANDER, MD, and
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The anastomosis of small blood vessels is most frequently performed by the conventional end-to-end (ETE) technique.^{1,2} In 1978, the end-in-end (EIE) anastomosis (telescoped or sleeve anastomosis) was reintroduced and reported to be technically simpler and faster to perform in small vessels than the ETE technique.^{3,4}

In a previous study using electromagnetic flowmetry, we found that the blood flow in small arteries was decreased by about 50% following EIE anastomosis.⁵ Angiograms made one day postoperatively also revealed stenosis in vessels anastomosed by the EIE technique but not by the ETE technique.

The purpose of the study reported here was to

compare ETE and EIE anastomoses using angiography.

MATERIAL AND METHODS

Thirty-five rabbits weighing between 2.5 and 5.0 kg each were used. They were kept on a standard pellet diet and given water ad libitum.

The rabbits were anesthetized with intravenous injections (60 mg/ml) of sodium pentobarbital (Mebumal Vet, ACO Läkemedel AB, Solna, Sweden); anesthesia was maintained with repeated injections. The renal, femoral, saphenous, and central ear arteries (average diameters as given below) were exposed and divided after the application of clamps. Anastomoses were performed by one of the following techniques.

End-to-End Anastomoses. Superfluous adventitia was removed for a distance of approximately 1 mm at each vessel end. Three stay sutures were placed 120° apart and a minimal number of interrupted sutures (3 to 5 in all) were placed between the stay sutures.

End-in-End Anastomoses. The EIE anastomoses were performed according to either the technique of Lauritzen³ or the technique of Meier.⁴ In

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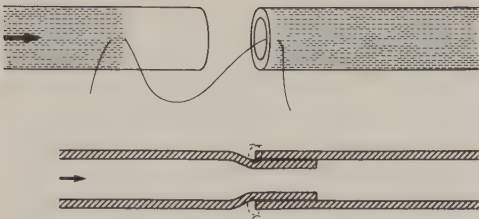


Figure 1. End-in-end anastomosis according to technique of Lauritzen. Arrows indicate direction of flow. Proximal vessel end is tucked into distal vessel lumen after sutures are tied.

both instances adventitia was excised for a distance of approximately 2 mm from the proximal vessel end and 1 mm from the distal vessel end.

Lauritzen Technique. In the Lauritzen technique (Fig. 1) two sutures were placed 180° apart. Each suture was begun by inserting the needle through the distal vessel wall close to the end and then passing the needle into the proximal vessel 2 mm from its end; only adventitia and part of the media were included in the needle pass through the proximal vessel. After the sutures were tied, the proximal vessel stump was inserted (telescoped) into the distal vessel lumen. To prevent serious leakage—more common in larger vessels—one or two sutures often had to be placed extraluminally midway between the first two sutures. When done properly, no foreign material is present in the vessel lumen.

Meier Technique. In the Meier technique (Fig. 2) two sutures were placed on opposite sides of the vessel. The needle was passed from the outside through the distal vessel wall 2 mm from the end and continued with a full-thickness bite close to

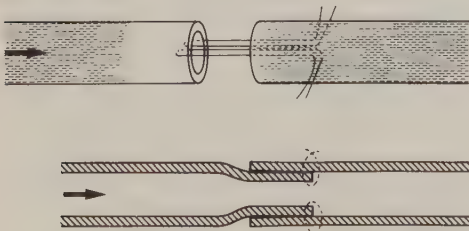


Figure 2. End-in-end anastomosis according to technique of Meier. Arrows indicate direction of flow. Proximal vessel end is pulled into distal vessel lumen by sutures when they are tied.

the end of the proximal vessel stump. The suture was completed by passing the needle through the distal vessel lumen and out through the wall close to the entry point. By tying the sutures, the proximal vessel stump is pulled inside the distal lumen. Care must be taken to place the sutures symmetrically or distortion of the vessel will result.

End-to-end anastomoses were only performed on the saphenous arteries and the central arteries of the ear (33 vessels; 0.8–1.2 mm in diameter). Lauritzen EIE anastomoses were performed on the same two types of arteries (33 vessels) and also on femoral arteries (5 vessels; 1.7–2.0 mm in diameter) and renal arteries (5 vessels; 2.0–2.7 mm in diameter). Meier EIE anastomoses were performed on the central arteries of the ear (5 vessels). All procedures were performed by the same surgeon (JBW).

Angiograms of the anastomoses were obtained in vivo with primarily enlarged pictures using projection angiography.⁶ The contrast medium was Isopaque (Nyegaard & Co., AS, Oslo, Norway) 350 mg I/ml. The following techniques were used:

1. Central arteries of the ear. A catheter was placed intra-arterially several centimeters proximal to the anastomosis.
2. Renal arteries. A catheter was inserted through the left femoral artery and positioned in the aorta at the origin of the left renal artery.
3. Femoral and saphenous arteries. A catheter was inserted through the contralateral femoral artery with its tip at the bifurcation of the aorta.

Angiograms were taken either one hour or 3, 7, 14, 30, or 90 days postoperatively. At each interval, five ETE and five EIE anastomoses (Lauritzen technique) were compared. Angiograms of the renal and femoral arteries after EIE anastomosis (Meier technique) were obtained only at the one hour interval.

The luminal areas of all of the anastomoses were calculated as a percentage of the luminal area of the vessel according to the formula:

$$L = D_a^2/D_v^2 \times 100$$

where L = the percentage of residual luminal area of the anastomosis, D_a = the smallest diameter of the anastomosis, and D_v = the vessel

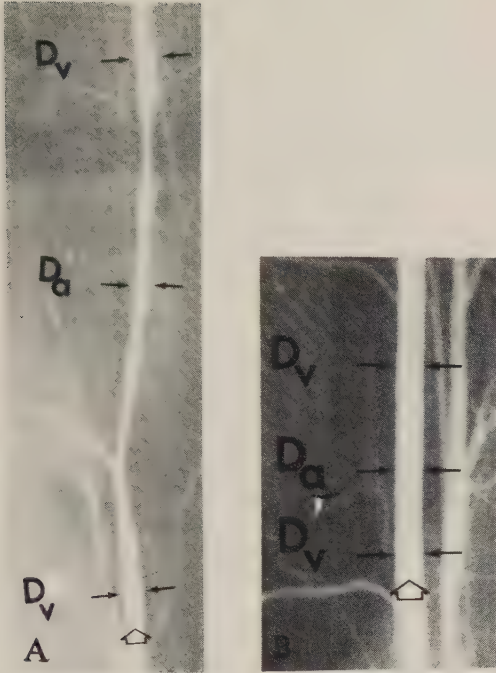


Figure 3. (A) End-to-end anastomoses one hour postoperatively and (B) after three months. Vessel diameter (D_v) was measured beyond areas with obvious dilation or spasm. D_a = diameter of anastomosis. Magnification: $\times 4.5$.

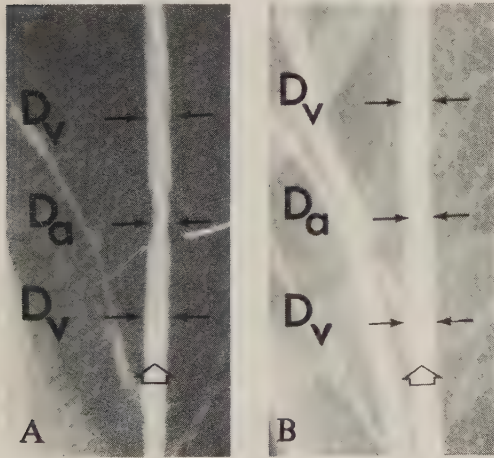


Figure 4. End-in-end anastomosis according to technique of Lauritzen (A) one hour after surgery, and (B) three months postoperatively. Results are typical, with considerable stenosis immediately after surgery and slight narrowing at site of anastomosis three months later. D_v = diameter of vessel; D_a = diameter of anastomosis. Magnification: $\times 4.5$.

diameter, which was measured within 2 cm both proximal and distal to the anastomosis and in segments of vessels with a uniform caliber of at least 1 cm (segments with spasm or dilation were excluded) (Figs. 3–5). The mean values of the proximal and distal diameters were used in the formula.

To preclude vasospasm, all of the vessels were treated with a 0.5 ml injection of lidocaine (Xylocain, 5 mg/ml, AB Astra, Södertälje, Sweden) before the angiograms were made. Measurements were made independently by two persons using adjustable dividers.

The results were compared statistically using Student's *t*-test.

RESULTS

Three ETE and six EIE anastomoses were found to be occluded and their results were therefore excluded. The occlusions occurred during the first and second postoperative weeks. Four EIE anastomoses in saphenous arteries occluded within one hour postoperatively.

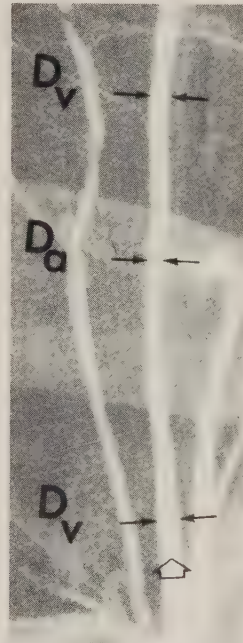


Figure 5. Meier end-in-end anastomosis one hour postoperatively. Result is typical, with moderate stenosis at site of anastomosis. D_v = diameter of vessel; D_a = diameter of anastomosis. Magnification: $\times 4.5$.

End-to-End Anastomoses in Saphenous Arteries and Central Arteries of the Ear. In the majority of vessels, ETE anastomosis did not change the luminal area except for one hour postoperatively, when a statistically significant ($P < 0.001$) decrease in luminal area was found (Figs. 3, 6). No aneurysms were noted.

Lauritzen EIE Anastomoses in Saphenous Arteries and Central Arteries of the Ear. With the Lauritzen EIE technique, all vessels showed considerable stenosis at the site of anastomosis up to seven days postoperatively (Figs. 4, 7). The mean luminal area of the anastomoses one hour postoperatively was 22% of the vessel luminal area. The diameters of the anastomoses increased later, but after seven days the luminal area of the anastomoses was only 35% of the vessel luminal area. Even after 90 days, four of five vessels still showed some degree of stenosis, the mean luminal area being 63% of the vessel luminal area.

The differences in luminal area between the ETE and EIE anastomoses were significant ($P < 0.01$) in all groups.

Lauritzen EIE Anastomoses in Femoral and Renal Arteries. The mean luminal area one hour postoperatively was 35% of the vessel luminal area in the femoral arteries (1.7–2.0 mm in diameter) and 48% of the vessel area in the renal arteries (2.0–2.7 mm in diameter) with the Lauritzen technique (Fig. 8).

Meier EIE Anastomoses in Central Arteries of the Ear. Angiograms obtained one hour postoperatively showed stenosis at the site of the anastomosis in all vessels with the Meier EIE technique (Figs. 5, 9). The mean luminal area was 39% of the vessel luminal area. Differences in the luminal area between this group and the corresponding group of Lauritzen EIE anastomoses were statistically significant ($P < 0.01$). No aneurysms were noted.

DISCUSSION

Angiograms of the ETE anastomoses one hour postoperatively showed a decrease in the mean luminal area of the anastomoses to 70% of the vessel luminal area. Since the diameters of the anastomoses and the vessel diameters proximal and distal to the anastomoses were very similar, however, vessel spasm may have been a major cause of the decrease in the ETE anastomoses (see Fig. 3A). On the other hand, in the case of the EIE anastomoses the diameters of the

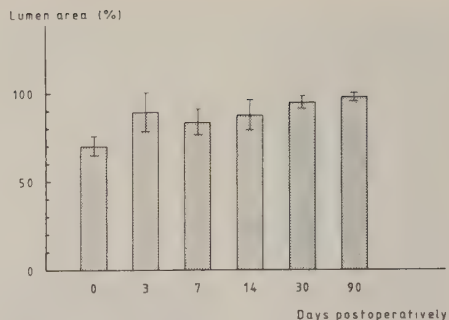


Figure 6. End-to-end anastomoses in arteries 0.8–1.2 mm in diameter. Each column represents mean luminal area of five anastomoses. Standard error of mean is indicated.

anastomoses were smaller than nearby vessel diameters and, hence, vasospasm could not account for the differences in luminal area (see Fig. 4).

During the first postoperative week, the EIE technique produced a decrease in the luminal area of more than 50%. This decrease is considered to represent "critical stenosis," which is in accordance with the results in our blood flow study on the same vessels.⁵ In another study, we followed the accumulation of isotope-labelled platelets after ETE and EIE anastomoses in the ear arteries of rabbits. During the first two hours postoperatively, there was no increase in platelet activity in the EIE anastomoses, indicating either no or very limited platelet accumulation.⁷ The stenotic effect caused by the EIE technique, using vessel ends of equal diameters, is therefore mainly due to doubling the vessel walls. In rabbits, dilated central arteries of the ear 1 mm in diameter have a vessel wall thickness of 0.15–

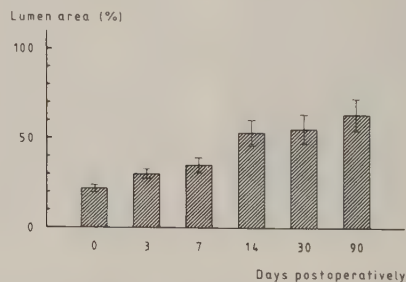


Figure 7. Lauritzen end-in-end anastomoses in arteries 0.8–1.2 mm in diameter. Each column represents mean remaining luminal area of five anastomoses. Standard error of mean is indicated.

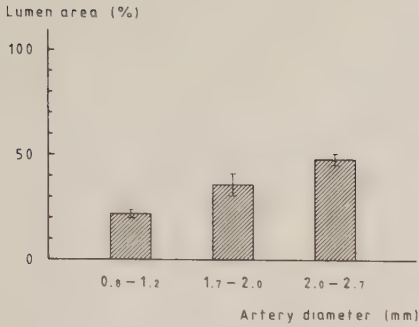


Figure 8. Lauritzen end-in-end anastomoses. Each column represents mean luminal area of five anastomoses in central arteries of ear (0.8–1.2 mm in diameter), femoral arteries (1.7–2.0 mm in diameter), and renal arteries (2.0–2.7 mm in diameter). Angiograms were obtained one hour postoperatively. Standard error of mean is indicated.

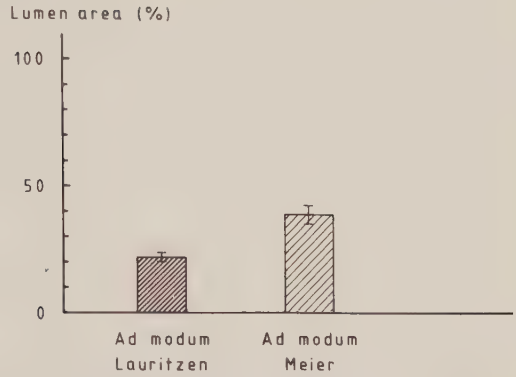


Figure 9. Lauritzen (left) and Meier (right) end-in-end anastomoses. Each column represents mean luminal area of anastomoses in central arteries of ear (0.8–1.2 mm in diameter). Angiograms were obtained one hour postoperatively. Standard error of mean is indicated.

0.20 mm and, hence, lumen diameters of 0.6–0.7 mm. Thus telescoping one vessel into the other decreases the effective lumen diameter to 0.2–0.4 mm. Since a reduction of the diameter by 50% results in a reduction of luminal area by 75%, the luminal area remaining should be about 25%, which is in accordance with our results (22% one hour postoperatively in the EIE anastomoses). It also follows that the stenotic effect of the EIE technique becomes less pronounced with increasing diameters.

The reasons for the gradual decrease in stenosis with the EIE technique are that the initial inflammatory reaction with edema di-

minishes and that the vascular walls are rebuilt (unpublished observations).

With the Meier EIE technique, the decrease in luminal area was less pronounced (39% versus 22%; $P < 0.01$). This requires further investigation and hence the present study will be extended.

In summary, in vessel ends of equal diameter, the EIE technique leads to stenosis in small vessels with gradual improvement during the following weeks and months. The present angiographic study shows that differences in the stenotic effect of the conventional ETE technique and EIE techniques are significant.

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III

ACCUMULATION OF ISOTOPE LABELLED PLATELETS IN SMALL ARTERIES AFTER END-TO-END AND END-IN-END ANASTOMOSES IN THE RABBIT

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Accumulation of isotope labelled platelets in small arteries after end-to-end and end-in-end anastomoses in the rabbit

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Summary—Microvascular anastomoses were performed under standardized conditions on the central artery of the ear in 8 rabbits. In all, 6 end-to-end and 5 end-in-end anastomoses were carried out. In 3 rabbits, one type of anastomosis was performed on each ear thus permitting simultaneous comparison of both types. Other than the local application of lidocaine, no anticoagulants or vaso-dilating agents were used.

³²P labelled platelets were injected intravenously 2 hours before microvascular anastomoses were performed. All end-to-end anastomoses showed a rapid increase in radioactivity immediately after removal of the vessel clamps. The activity reached a peak some 300–600% above the initial value after approximately 30 minutes and then decreased. The patent vessels in the end-in-end group showed no increase in platelet activity and the difference between the two groups was significant during the first two hours. The results are interpreted as showing that platelet accumulation in patent vessels is more pronounced in end-to-end than in end-in-end anastomoses.

Introduction

The occurrence of thrombotic occlusions at the site of an anastomosis is a major problem in microvascular surgery (Acland, 1973). Subendothelial structures are frequently exposed at vascular clamp sites and always in the vicinity of an anastomosis. Scanning electron and light microscopy have shown that these damaged areas become covered with platelets (Nightingale *et al.*, 1980). Platelet aggregation is necessary to prevent blood leakage, by sealing defects. Platelet accumulation can, however, exceed desirable levels and growing platelet aggregates then become a threat to unimpeded blood flow. Attention has recently been focused on the interrelations between the vascular wall, platelets and pro- and anti-aggregatory substances (Wallace *et al.*, 1980).

Microvascular anastomosis according to the end-to-end (ETE) technique is well established (Acland, 1979; Buncke *et al.*, 1975). In 1978, two different models of performing an end-in-end (EIE) anastomosis (sleeve, telescope anastomosis) were reported to be safe and technically simpler methods of uniting small vessels (Lauritzen, 1978; Meier, 1978).

In a previous study we used electromagnetic flowmetry (EMF) and found that the mean blood flow in small arteries (0.8–1.2 mm) was almost unchanged after ETE anastomosis, but was reduced to approximately 50% of the pre-operative value following EIE anastomosis. Microangiography showed marked stenosis after EIE anastomosis, but not after ETE anastomosis (Wieslander and Åberg, 1980). In the present work the accumulation of platelets after anastomosis has been studied using a radioactive tracer technique.

Material and methods

Eight rabbits, weighing between 3.0–4.0 kg, were used. They were kept on a standard pellet diet and given water *ad libitum*.

All anastomoses were performed on the central artery of the ear. This artery is readily accessible and of a suitable diameter (0.8–1.2 mm). In 3 rabbits an ETE anastomosis was performed on one ear and an EIE anastomosis on the other ear, thus enabling platelet radioactivity recordings to be made simultaneously in both ears. In a further 5 rabbits, ETE anastomosis was performed in 3

ACCUMULATION OF ISOTOPE LABELLED PLATELETS IN SMALL ARTERIES

and EIE anastomosis in 2 rabbits. The same surgeon (JBW) performed all the operations.

The temperature of the animals was maintained at $39.8^{\circ}\text{C} \pm 0.2^{\circ}$ during the entire procedure by a thermostatically regulated lamp and an electric heating pad. No anticoagulants or vasodilating agents were used, other than the local application of lidocaine (Xylocaine, 10 mg/ml, AB Astra, Södertälje, Sweden). Tyrode's solution without magnesium sulphate was used as irrigant. Magnesium sulphate was omitted because it has been reported to decrease platelet adhesiveness (Acland, 1972). Dermalon sutures (10-0, Davis and Geck) were used in all anastomoses.

Labelling of platelets

One hundred and fifty ml of homologous whole blood was used. Platelets were isolated and labelled with ^{32}P according to Abrahamsen (1968) with the following modifications:

- (i) Instead of ^{51}Cr , ^{32}P was used.
- (ii) To enhance labelling efficiency, platelets were incubated with 25 MBq ^{32}P for 2.5 hours at 37°C .

To evaluate the stability of ^{32}P labelled platelets in the blood, samples were taken after 30 minutes and then at hourly intervals up to 6 hours after infusion. The radioactivity was measured in a well-counter (using a NaI (Tl) crystal). Three blood fractions—platelets, plasma and the corpuscular fraction—were measured separately. The total blood activity was calculated from these values.

Following anastomosis, the ^{32}P activity was followed for 2–4 hours, values being registered immediately after removal of the vascular clamp and subsequently every 15 minutes.

Registration of radioactivity

GM tubes (Philips type 18507) were used to detect the beta radiation from ^{32}P labelled platelets. The GM tubes were screened with rectangular blocks of lead with 6 mm minimum wall thickness. The unscreened end of the GM tube was positioned immediately over the exposed central artery. Background was further reduced by placing the vessel in a slit made in a rectangular lead plate 6 mm thick (Fig. 1). The

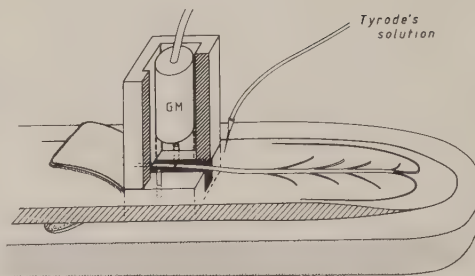


Fig. 1 The set-up used in these experiments. GM=Geiger Müller tube.

lead block and the plate were joined together by metal pins, but they could easily be separated and repositioned.

The signals from the GM tubes were shaped using locally-built discriminators. These provided pulses suitable for counting in scalers (Selectronix Scaler-Timer Model 47-21) and for connecting to a rate-meter (Selectronix Analyzer Model 45-22). The output of the latter was monitored continuously on a pen recorder. The number of pulses per 100 seconds was recorded on the scaler at regular intervals. Two independent counting-channels were used, so that measurements could be made simultaneously on both ears of one rabbit.

The numbers of counts recorded over the vessels were evaluated statistically using Student's test for paired values.

Procedure

The rabbits were anaesthetized intravenously with sodium pentobarbital (Mebumal Vet., 60 mg/ml, ACO Läkemedel AB, Solna, Sweden) and anaesthesia maintained by intermittent injections of the same compound.

Two flaps measuring 3×2 cm were raised on either side of the central vessels of the ear. The vein was ligated, the artery isolated and its side branches ligated. To counteract induced vasospasm, small amounts of lidocaine were applied locally. The wound area was continuously irrigated with Tyrode's solution. The exposed artery was placed in the slit in the lead plate and the GM tube in its lead block positioned immediately over it (Fig. 1). Care was taken to remove any blood present. The

operative field was then covered with a thin plastic film.

Following platelet infusion, the activity of the ^{32}P isotope was immediately registered on one or both ears. To be able to separate vessel activity from background activity, a lead plate was placed over the artery, thus screening it from the counter. This was repeated several times during the measurements. The activity in the vessels was monitored for 2 hours before performing the anastomosis. The activity levels quickly reached a steady state.

ETE anastomosis

The GM tube was removed and an Acland double microvascular clamp placed on the artery. The artery was divided and superfluous adventitia excised for a distance of 1–2 mm at each end of the cut vessel. Three stay sutures were placed at 120° intervals and a minimal number (3–5) of interrupted sutures placed among them.

EIE anastomosis

The vessel was placed in a double microvascular clamp as above and divided (Fig. 2). Adventitia was removed for a distance of approximately 2 mm from the proximal end and approximately 1 mm from the distal end. Two sutures were placed as follows: The first suture was started by inserting the needle through the adventitia and part of the media 2 mm from the end of the proximal stump. The suture was then continued with a full thickness bite through the wall of the distal stump close to its end. The second suture was placed in the same manner on the opposite

EIE ad modum Lauritzen

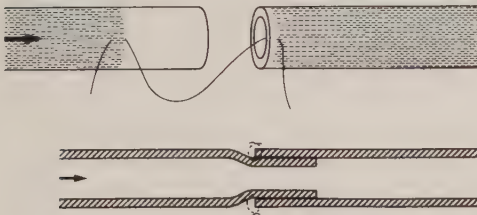


Fig. 2 End-in-end anastomosis ad modum Lauritzen. The arrow points out the direction of blood flow. (see text).

side. Next, the proximal stump was inserted into the distal lumen. To prevent serious leakage, third and fourth sutures had often to be placed extraluminally midway between the 2 sutures. When done properly, no foreign material entered the vessel lumen.

Results

Platelet labelling

The activities in the platelet and corpuscular fractions slowly decreased as did the total blood activity. The curves ran nearly parallel (Fig. 3). The plasma activity was normally low (1–4%). The measured value of radioactivity in the platelet fraction (8 animals) was $65\% \pm 3$ of the total blood activity after 0.5 hours (see discussion). The decrease in platelet activity between 2 and 6 hours after infusion was in the range 61–39%.

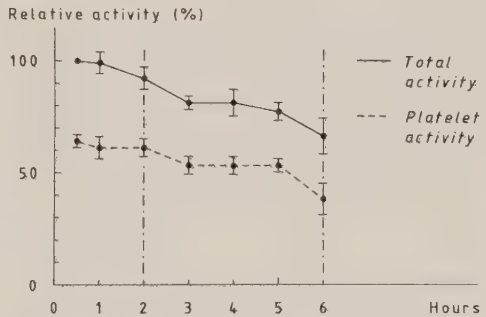


Fig. 3 The variations of ^{32}P activities in whole blood (—) and platelet fraction (---) as a function of time after injection. The standard errors are as indicated. The vertical lines denote the interval during which measurements were made over the anastomotic segment.

Activity over the arterial anastomoses

All values of radioactivity were calculated as percentages of the steady state values, registered before performing the anastomoses.

ETE anastomoses (Fig. 4)

In all ETE anastomoses, the activity increased rapidly immediately after the removal of the vascular clamps, reaching a maximum value of $414\% \pm 94$ ($p < 0.01$) after about 30 minutes. Platelet activity then decreased—at first rapidly and then more slowly.

EIE anastomoses (Fig. 4)

The patent vessels in the EIE anastomoses showed no increase in platelet activity.

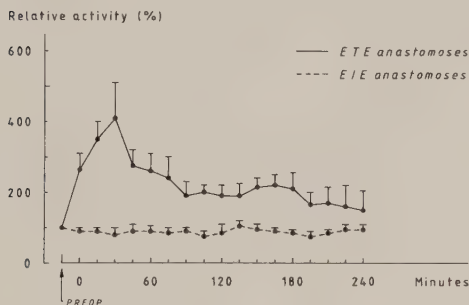


Fig. 4 Activities of ^{32}P labelled platelets in ETE (—) and EIE (---) anastomoses as a function of time after release of anastomotic clamps.

Thrombotic vessels (Fig. 5)

One anastomosis in the ETE group and one in the EIE group developed occluding thrombosis, as confirmed by patency tests. These thrombotic vessels showed the same initial pattern as the ETE group but then remained at high levels.

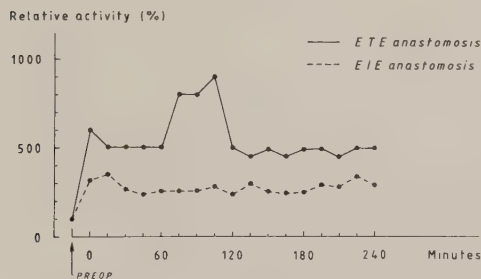


Fig. 5 Activities of ^{32}P labelled platelets in an ETE (—) and an EIE (---) anastomosis with thrombotic occlusion as a function of time after release of the vascular clamp.

Comparison of simultaneous ETE-EIE anastomoses (Fig. 6)

In case I, both the ETE and EIE anastomoses follow the general pattern described above (Fig. 4). In case II, the measured activity of the ETE anastomosis showed a second peak 2–3 hours

after anastomosis, followed by a rapid decrease. The blood flow was checked several times and seemed by visual inspection to be good. In case III, the activity over the EIE anastomosis increased as did the corresponding ETE activity, but remained high due to occluding thrombosis.

Discussion

The labelling of platelets with the gamma emitter ^{51}Cr is a well-known method (Abrahamsen, 1968). However, in this work, the vessel segment in question (diameter 1 mm, length 20 mm) contains only approximately 0.0001 of the total blood volume of the rabbit and we were unable to separate vessel activity from background using this isotope as label. Using the beta-emitting isotope ^{32}P , the platelet activity in the vessel segments could be registered and separated from the background (Ebbe *et al.*, 1965). This is mainly due to the short-range nature of the beta-particles as compared to gamma radiation. In the study of the isotope activity in the platelet fraction, the mean value of the first samples—taken 0.5 hours after infusion—was 65% of the total activity. Most of the remainder was due to platelets in the corpuscular fraction and only a few percent to activity in the plasma fraction. These results reflect the usual difficulties in separating platelets from the corpuscular fraction. All separations were made by the same laboratory assistant and the data are consistent with the separation errors being approximately constant. The isotope activity in the platelet fraction decreased from 61% two hours after infusion to 39% four hours later. These activity values are expressed as percentages of the values 0.5 hours after infusion of the labelled platelets. The activity of the platelet fraction decreased by 36% during the 4 hour postoperative period. The loss in platelet activity was probably due to excretion of free ^{32}P or transfer to other body compartments.

The results clearly show a significant difference between platelet accumulation in ETE as compared to EIE microvascular anastomoses during the first two hours following removal of the vascular clamps. There was no accumulation of platelets in EIE anastomoses, a finding which is not in accordance with results from light and scanning electron microscopy, in which the gap at the end of the telescoped vessel was found to be filled with thrombotic material. If we take

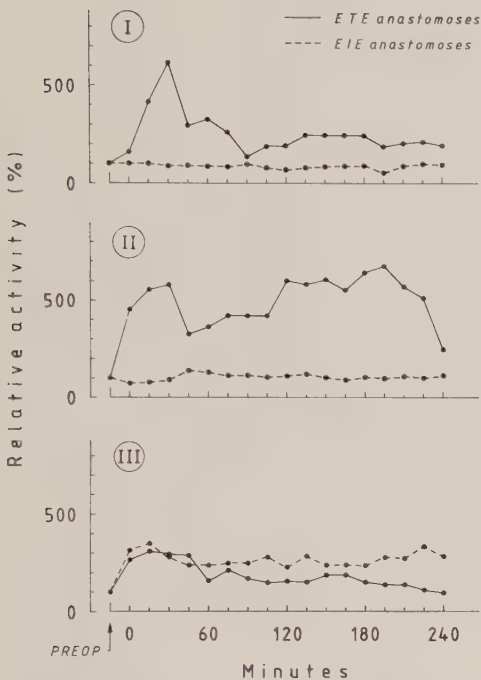


Fig. 6 Activities of ^{32}P labelled platelets in ETE (—) and EIE (---) anastomoses during simultaneous registration as described in the text. All vessels were patent except the EIE anastomosis in case III.

into consideration the decrease of activity in the platelet fraction and of blood volume in the stenotic EIE anastomoses—as found in our previous study (Wieslander and Åberg, 1980)—there is a small increase in activity, indicative of a modest platelet accumulation in the EIE group. The most plausible reason for the high activity at the ETE anastomotic site is the large number of platelets required to seal leaks. Another possible reason is the presence of intraluminal foreign material in ETE but not EIE anastomoses. When an EIE anastomosis remains patent in such small arteries, blood flow velocities increase through the stenotic segment and platelet accumulations may be washed away more rapidly (Arfors *et al.*, 1968).

The rapid decrease in activity observed after a few hours indicates that intravascularly located platelets are washed away by the blood stream. The possibility that some altered platelets

suddenly release their contents of ^{32}P cannot, however, be completely excluded.

Since, except in the thrombotic cases, no accumulations of platelets were observed after EIE anastomoses the 50% decrease in blood volume flow observed earlier (Wieslander and Åberg, 1980) can probably be ascribed to the stenotic effect of telescoping vessel ends of equal diameters. Using the ETE technique, there is considerable accumulation of platelets but this ordinarily affects blood volume flow to a much smaller degree.

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IV

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MICROARTERIAL END-IN-END (SLEEVE) AND
END-TO-END ANASTOMOSES

Jan B. Wieslander, MD and Alf Rausing, MD

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ABSTRACT

Twenty-five microvascular end-in-end and 21 end-to-end anastomoses were performed on the central arteries (diameters 0.8-1.2 mm) of rabbit ears. The appearance of longitudinal specimens taken after intervals of 1-2 hours and 1, 3, 7, 14, 30 and 90 days was studied microscopically.

Between 1-2 hours and 14 days, all end-in-end anastomoses showed considerable narrowing of the telescoped segment and platelets had accumulated at the sleeved vessel end. The vascular walls of the telescoped segment were thickened distally and the internal elastic laminae folded in the longitudinal direction due to contraction. Extensive loss of endothelium was observed both around sutures and distally in the telescoped segments. The stenosis initially noted in telescoped vessels gradually decreased due to atrophy of the medial layers in the double vascular walls. Following the organization of the platelet aggregates, subsequent endothelialization was completed between 7 and 14 days. After 2 weeks, neointimal hyperplasia was frequently found inside fibrotic medial and adventitial layers. In the early postoperative period, platelets covered exposed subendothelial structures and sealed defects in the vessel walls of all end-to-end anastomoses. During the first few postoperative weeks, the medial layers disintegrated and were replaced by increasing fibrosis. Reendothelialization was completed between 7 and 14 days, whereafter gradual intimal hyperplasia became significant. Thus, platelet accumulation, organization and subsequent endothelialization differed between the two techniques. No aneurysms were found in either group.

INTRODUCTION

The healing of conventional microvascular end-to-end (ETE) anastomoses has been studied in several works (1,2,3,4). Histological studies of end-in-end (EIE, telescope, sleeve) anastomoses (5,7) are few (6,7,8) and no simultaneous comparative study of both techniques has as yet been reported in the literature. The aim of the present work was therefore to study the healing process in small arteries (diameters 1 mm) using these techniques.

MATERIAL AND METHODS

Rabbits, male and female, weighing between 2.5-4.0 kg, were kept on a standard pellet diet and given water ad libitum.

Twenty-five EIE and 21 ETE anastomoses were performed on the central arteries (diameters 0.8-1.2 mm) of the ear. Dermalon 10-0 sutures (Davis & Geck) were used in all anastomoses.

Operative procedure

All rabbits were anesthetized with intermittent injections of sodium pentobarbital (Mebumal Vet. 60 mg/ml, ACO Läkemedel, Solna, Sweden).

Skin flaps were raised over the central vessels of both ears and the arteries prepared over a distance of 15-20 mm, whereby tension arising from use of the sleeve technique was avoided. After exposure, the arteries were placed in double microvascular clamps and divided. Anastomosis was then performed using one of the following techniques:

End-to-end anastomosis: (9)

Superfluous adventitia was excised at both ends of the cut vessel. Three stay sutures were placed 120° apart and 3-5 interrupted sutures placed among them. The vascular clamp was remo-

ved, anastomotic patency checked and the skin flap sutured in its previous position.

End-in-end anastomosis:

The technique used was that described by Lauritzen (1978) (5). The adventitia at the proximal vessel end was carefully removed for a distance of approximately 2 mm and excessive adventitia at the transected distal vessel end excised. Two sutures were placed 120° apart (a full thickness bite being taken through the distal vessel end and a partial bite through the proximal stump 2 mm from the end), care being taken not to enter the new lumen with the suture material. The sutures were tied and the proximal vessel end was then telescoped into the distal lumen. When needed to stop leakage of blood, one or two additional sutures were placed between the main sutures. After releasing the double vascular clamp, patency was checked and the skin incision closed.

Preparation of vessel specimens:

Anastomosed vessels - 3 in each group - were taken for study after periods of 2 hours, and 1, 3, 7, 14, 30 and 90 days post-anastomosis. A small catheter (PA 50-60) was placed in the central artery of the ear 2-3 cm proximal to the anastomosis. Small amounts of Ringer acetate (ACO Läkemedel, Solna, Sweden) were used to prevent clotting in the catheter. Otherwise, perfusion of the vessel was avoided. All vessels were then perfused for 15 minutes at a constant pressure of 120 mm Hg with a special fixative (10) (composition: 0.2 M phosphate buffer 400 ml + 50% glutaraldehyde 50 ml + 20% dextran T70 95 ml + dist H₂O 455 ml). This fixative made it possible to use the specimens also for scanning electron microscopy. The animals were killed at the start of perfusion.

The skin flap was raised after fixation and the anastomosed artery could easily be separated from its surrounding tissues. To avoid kinking, the vessel samples (20 mm in length) were mounted in a double Acland microvascular clamp (11) and kept in the same fixative for 12 hours. The samples were then washed 4-5

times in Millonig's phosphate buffer and kept immersed in the same fluid until histological processing began.

The specimens were dehydrated and embedded in paraffin. Longitudinal 4 μ thick step sections were cut and stained with Weigert-van Gieson stains. Four additional EIE specimens prepared after 7 days were stained with phosphotungstic acid haematoxylin for fibrin and Giemsa and Gordon's and Sweet's reticulin methods.

RESULTS

Thrombosis

2 ETE and 2 sleeve anastomoses were occluded by thrombi. The occlusions occurred, following both techniques, at 1-2 hours and at 3 days postoperatively.

2 partial vessel occlusions following the ETE technique were observed at 1-2 hours and at 7 days post-anastomosis. Using the sleeve technique, 4 partially occluding thrombi at the telescoped vessel end were found at 1-2 hours (one) and at 7 days (three) postoperatively.

Results concerning the healing of both types of anastomosis at various times are summarized in the table IV:5

Extra information is also to be found in the captions to the figures, which show representative segments.

		1-2 hours	1 day	3 days	7 days	14 days	30 days	90 days
Lumen	EIE (sleeve)	Pronounced narrowing	Decreasing stenosis		Moderate narrowing Extensive reendothelialization	Slight narrowing Reendothelialization complete	Similar appearance	Lumen uniform
	ETE	Normal width	Normal width	Reendothelialization begun	Reendothelialization almost complete	Reendothelialization complete		
Platelet aggregates	EIE (sleeve)	At telescoped end	Fibroblast invasion Organization begun	Massive fibroblast infiltration. Loose connective tissue formation. Organization	Organization complete	Similar appearance	Transformation complete	
	ETE	Between transected ends + intraluminaly						
Vessel wall	EIE (sleeve)	Occasionally bleeding + oedema intramurally	Disintegration Leukocyte infiltration	Fibroblast invasion Atrophy of external medial layers	Fibrosis of external layers. Endothelialization of pockets between walls	External wall healed by fibrosis. Hyperplastic neointima distal to anastomosis. Giant cells around sutures.	Progressive intimal hyperplasia	Stagnant intimal hyperplasia proximally and distally to anastomosis. Double walls still remaining
	ETE							
								External fibrosis. Stagnant intimal hyperplasia proximally and distally to anastomosis. No continuity between fragmented elastic laminae

CHARACTERISTIC MICROSCOPICAL FINDINGS AT VARIOUS TIMES

* Pockets were found between the double vascular walls in almost all EIE anastomoses up to 7 days post anastomosis.
Endothelialized pockets were found in 50% of EIE anastomoses between 7 and 90 days.

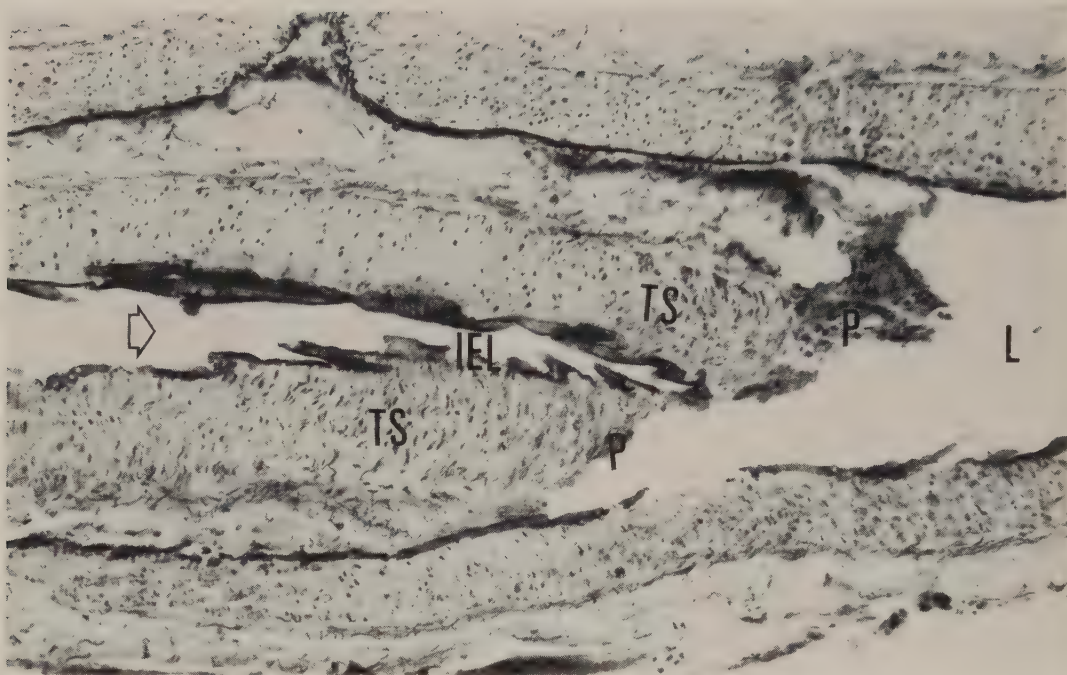


Fig. 1. End-in-end anastomosis at 1-2 hours. Pronounced narrowing of lumen. Platelets (P) adhere to the raw vessel end of the telescoped segment (TS). The internal elastic lamina (IEL) is folded and the distal part of the sleeved segment shows signs of thickening. Lumen (L). The open arrow (◻) indicates the direction of blood flow. Weigert-van Gieson Stain. (200 x).

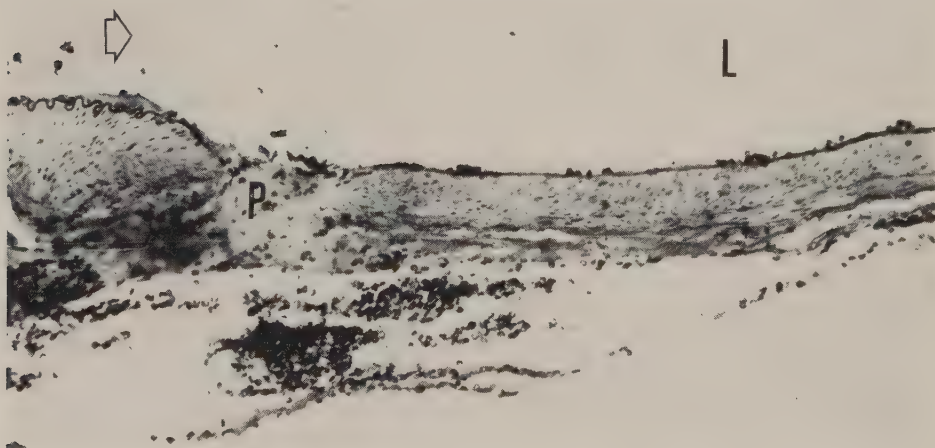


Fig. 2. End-to-end anastomosis at 1-2 hours. Platelet aggregates (P) seal defects between the vessel ends. The open arrow (◻) indicates the direction of blood flow. Lumen (L). Weigert-van Gieson Stain. (240 x).

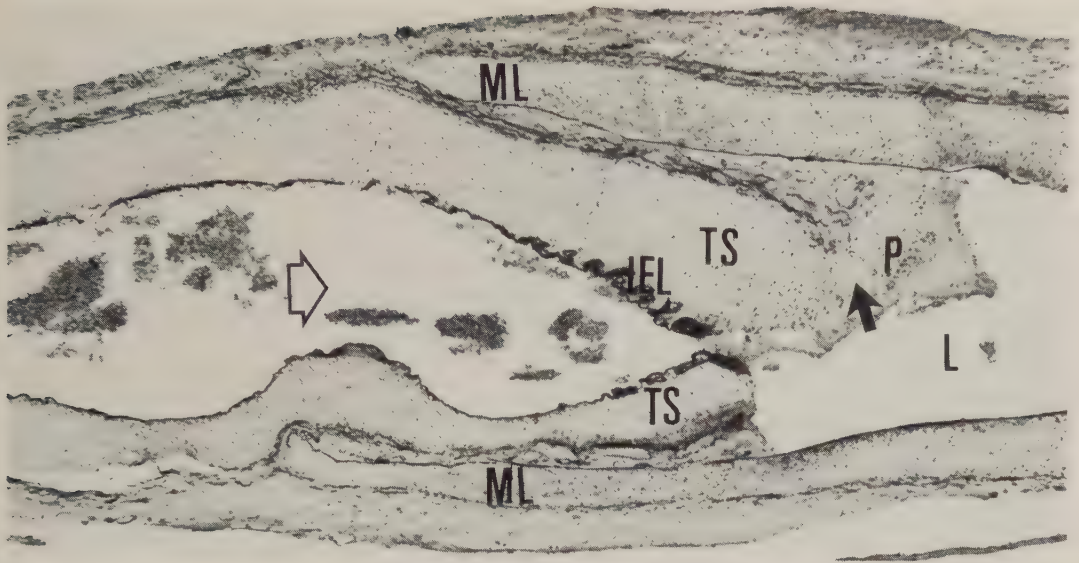


Fig. 3. End-in-end anastomosis at 3 days. Contraction of the telescoped segment (TS) causes thickening distally and folding of the internal elastic lamina (IEL). The medial layer (ML) of the external vessel is thinner in the anastomotic region. The platelet aggregate (P) at the end of the sleeved segment has been invaded by cells and organization has begun (■). Lumen (L). The open arrow (↗) indicates the direction of blood flow. Weigert-van Gieson Stain. (90 x).

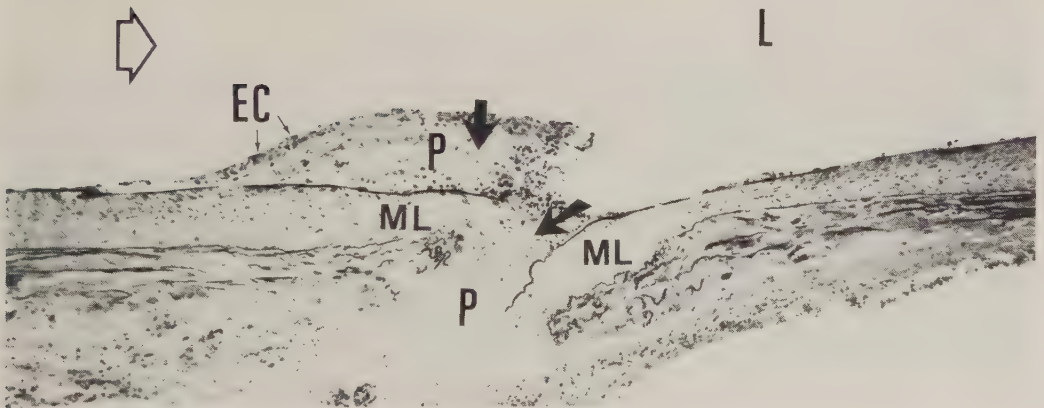


Fig. 4. End-to-end anastomosis at 3 days. The medial layers (ML) close to the anastomosis (transected ends) have disintegrated. Platelet aggregates (P) located both intraluminally and between the anastomosed vessel ends have been invaded by cells and organization has begun (■). New endothelial cells (EC) cover part of the intraluminal platelet aggregate. Lumen (L). The open arrow (↗) indicates the direction of blood flow. Weigert-van Gieson Stain. (90 x).

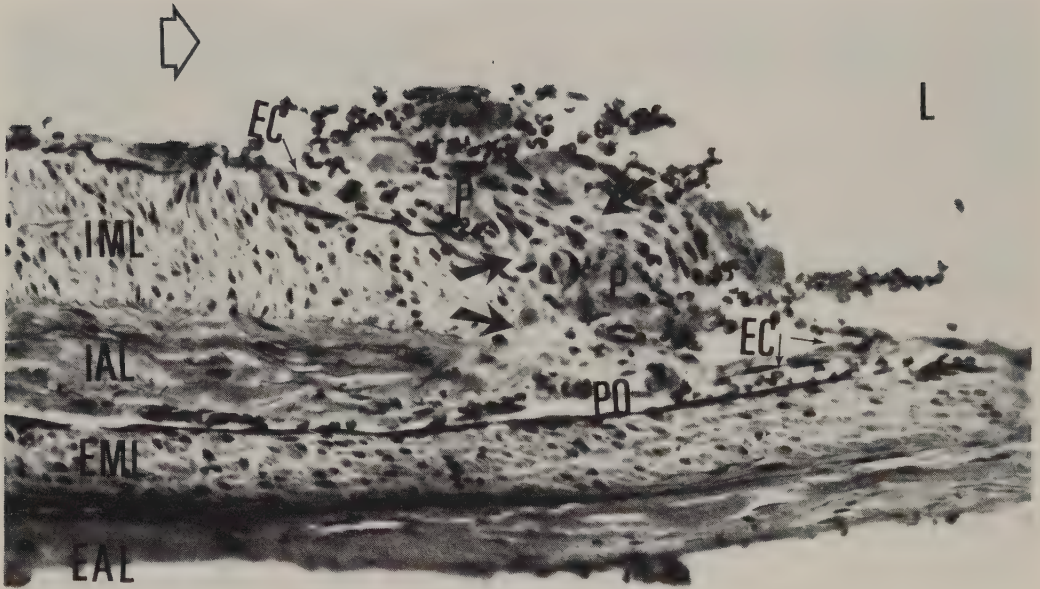


Fig. 5. End-in-end anastomosis at 7 days. The distal part of the telescoped segment and the adherent platelet aggregate (P) are heavily infiltrated with cells and transformation to loose connective tissue (▀) has begun. Pockets (PO) seen between the two vascular walls are partly covered by young endothelial cells. New endothelial cells also cover part of the anastomotic region. Internal medial layer (IML). Internal adventitial layer (IAL). External medial layer (EML). External adventitial layer (EAL). Lumen (L). The open arrow (◁) indicates the direction of blood flow. Weigert-van Gieson Stain. (380 x).

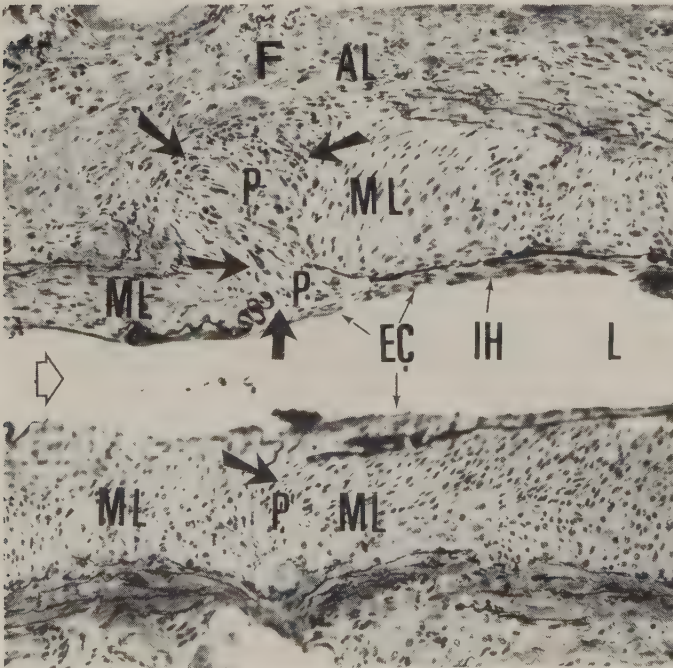


Fig. 6. End-to-end anastomosis at day 7. Pronounced cell invasion of platelet aggregates (P). Transformation of these aggregates (P) with the formation of loose connective tissue (▀) has begun. Endothelial cells (EC) cover the internal anastomotic region. Intimal hyperplasia (IH) has also formed. Disintegration of the medial layers (ML) close to the transected vessel ends is also observed. Fibrous tissue (F) provides continuity of the adventitial layers (AL). Lumen (L). The open arrow (◁) indicates the direction of blood flow. Weigert-van Gieson Stain. (180 x).



Fig. 7. End-in-end anastomosis at day 14. Endothelialization (EC) is complete. Intimal hyperplasia (IH) is conspicuous and 5-6 cell layers thick. Atrophy, thinning and fibrosis (F) of the medial layers (ML) are found at the anastomotic region. Lumen (L). The open arrow (◻) indicates the direction of blood flow. Weigert-van Gieson Stain. (110 x).

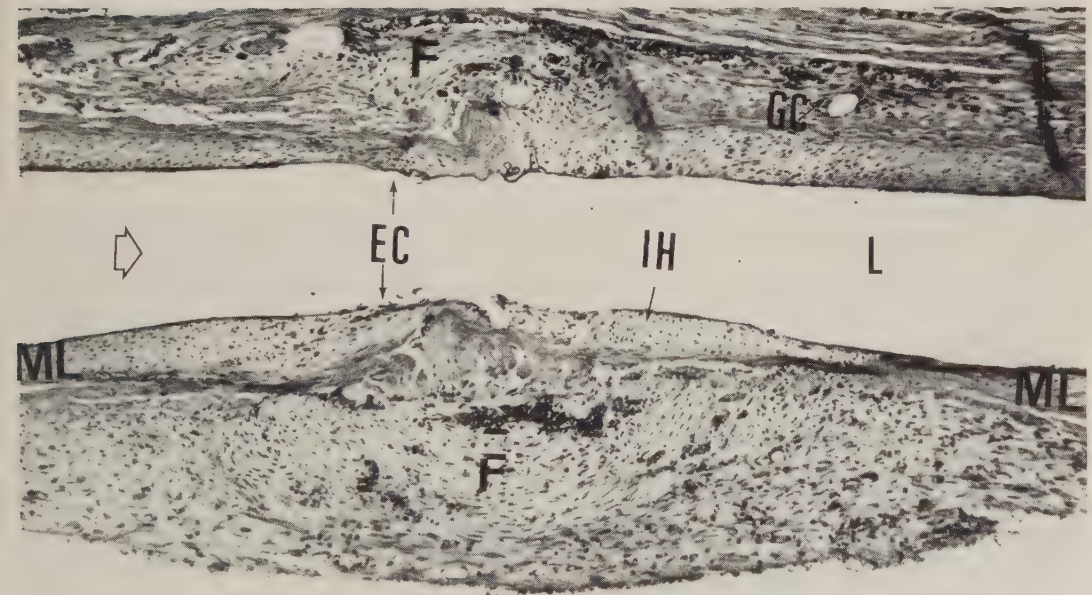


Fig. 8. End-to-end anastomosis at day 14. Reendothelialization (EC) is complete. The vascular walls close to the anastomosis are atrophic and show fibrosis (F). A hyperplastic neointima (IH) makes the lumen diameters more uniform. Foreign body giant cell formation (GC) can be seen around sutures. Medial vascular layer (ML). Lumen (L). The open arrow (◻) indicates the direction of blood flow. Weigert-van Gieson Stain. (130 x).



Fig. 9. End-in-end anastomosis at day 90. The double vascular walls are clearly visible. The internal surface consists of thick hyperplastic neointima (IH) making the discontinuity between the two vessels smooth. Fibrous tissue (F) replaces most of the atrophic external vascular walls. Internal medial layer (IML). External medial layer (EML). Lumen (L). The open arrow (◻) indicates the direction of blood flow. Weigert-van Gieson Stain. (130 x).

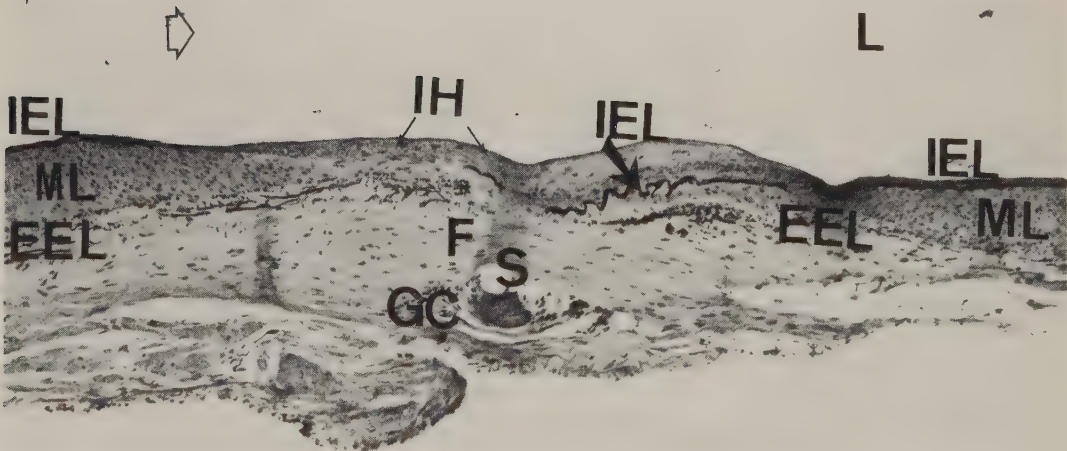


Fig. 10. End-to-end anastomosis at day 90. The internal part of the vascular wall consists of multilayered intimal hyperplasia (IH). Outside the neointima the vascular wall is atrophic with fibrosis (F). No continuity between the external (EEL) or internal elastic laminae (IEL). Foreign body giant cells (GC) are seen around sutures (S). Lumen (L). The open arrow (◻) indicates the direction of blood flow. Weigert-van Gieson Stain. (130 x).

DISCUSSION

Fixation of the vessels was performed with a new technique, thus avoiding perfusion with solutions such as saline solution which may cause endothelial damage (12). The composition of the perfusion fixative has been developed so as to give a minimum of fixation artefacts, when studied with scanning electron microscopy (10). In addition, the animals were alive until the commencement of perfusion. Fixation under pressure (120 mm Hg) is physiologically more satisfactory, gives fewer artefacts and facilitates the mounting of double microvascular clamps and subsequent sectioning. As has been reported by various authors, the preparation of small vessels in fibrotic tissue is difficult and time-consuming. The method described here with fixation prior to the preparation of the vessels simplifies the procedure and has the advantage of reducing the risk of damaging the vessels.

Comparison of the histological appearance of small arteries after EIE and ETE anastomoses reveals obvious differences in the repair processes taking place. Immediately after anastomosis, the EIE technique causes considerable narrowing due to doubling of the vascular walls and to contraction of the telescoped segment (Fig. 1). This accounts for the severe stenosis in the early postoperative period previously observed with angiography (Wieslander & Åberg, 1982) (13).

Gradual increase in the lumen diameter could be due to the fact that the medial layers in both vascular walls show considerable atrophy after 3-7 days. The less pronounced stenosis in larger arteries after sleeving depends on the fact that the vessel walls are proportionately thinner in comparison to the lumen diameter.

Platelets accumulate after both types of anastomosis. In EIE anastomoses, the cut end of the telescoped segment is covered by platelets, sometimes forming an aggregate bridging the discontinuity to the external vessel intima (Figs. 1, 3 and 5). In ETE anastomoses, however, platelets adhere to and seal defects between the vessel ends and the suture holes (Figs. 2, 4 and 6).

This requires more platelets than in EIE anastomoses, explaining the registration of higher activities of isotope-labelled platelets in ETE anastomoses than in EIE anastomoses, reported in a recent work (Wieslander, Åberg and Dougan, 1982) (14). The present material provides an excellent opportunity of studying the fate of platelet aggregates located at the ends of telescoped segments (Figs. 3 and 5). Between 1 and 3 days post-anastomosis, these aggregates are invaded by fibroblasts coming from the sleeved vessel end. Platelet aggregates are organized and rebuilt with the formation of loose connective tissue, which resembles the medial layer of the wall and forms a bridge at the step between the two vessel lumens. Reendothelialization occurred on top of the organized aggregates. Further studies to elucidate the mechanisms of cell invasion, transformation and endothelialization of platelet aggregates will be performed.

A comparison of 90 day results after end-in-end and end-to-end microarterial anastomoses shows similar results for the repair process with neointimal hyperplasia inside the internal elastic membrane and external to this heavy fibrosis involving medial and adventitial layers. Using the telescope technique, the double vascular walls remained clearly visible throughout the entire period studied.

CONCLUSION

The observations on longitudinal profile specimens using light microscopy explain the severe luminal narrowing caused by the sleeve technique in small arteries as being due to:

- 1) The doubling of the vascular walls using vessel ends of equal diameters.
- 2) Contraction of the sleeved vessel segment.

The gradual increase in the initially decreased luminal area following the sleeve technique depends mainly on severe atrophy of the medial layers in the double vascular walls in the weeks and months following the operation.

The incidence of occluding thrombi was the same for both techni-

ques, but the high rate of partial occlusions at 7 days using the sleeve technique indicates an increased risk of late anastomotic failure.

The results suggest that the sleeve technique should be reserved for larger vessels or be used in small vessels only when a favorable discrepancy exists between the diameters of the vessel ends.

ACKNOWLEDGEMENTS

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V

**ENDOTHELIALIZATION FOLLOWING END-TO-END AND
END-IN-END (SLEEVE) MICROARTERIAL ANASTOMOSES**

A scanning electron microscopical study

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MICROARTERIAL ANASTOMOSES
A scanning electron microscopical study

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ABSTRACT

Eighteen microvascular end-to-end and eighteen end-in-end anastomoses were performed on the central arteries (diameters 0.8-1.2 mm) of rabbit ears. Specimens taken after intervals of 1-2 hours and 3, 7, 14, 30 and 90 days were studied using a scanning electron microscope.

At 1-2 hours platelets and to a minor extent erythrocytes and fibrin had accumulated in defects between the vessel ends on sutures and at the suture holes of all end-to-end anastomoses. This had also occurred at the distal end of the telescoped segment in all end-in-end anastomoses. At 3 days endothelial desquamation at anastomotic sites and clamp sites was found in both end-to-end and end-in-end anastomoses. The exposed subendothelial surface was covered with a carpet of platelets and frequently numerous leucocytes were found. Between 3 and 7 days, new endothelial cells appeared at the junction between the vessels in end-to-end anastomoses and in all areas damaged by vascular clamps. At the ends of the sleeved segments reendothelialization was observed over platelet aggregates. Between 7 and 14 days neo-endothelialization seemed to be virtually completed in both types of anastomosis. In the vicinity of the anastomoses, the appearance of the new endothelium was frequently abnormal with irregularly formed cells. Characteristic clefts and holes were seen between these abnormal (pleomorphic) endothelial cells. Histological findings from the same vessel speci-

mens show that areas with abnormal endothelialization correspond to segments with intimal hyperplasia. Such areas were found in specimens throughout the entire period studied. When the telescope technique was used, the gap between the two vessels could still be seen in specimens up to 90 days post-anastomosis.

INTRODUCTION

Endothelial regeneration following microvascular anastomosis has been the subject of several investigations (1,2,3,4,5,6,7). The endothelialization of an end-in-end (EIE, sleeve) anastomosis in rat arteries and veins was studied up to 3 weeks postoperatively by Lauritzen in 1978 (8,9). A comparison of the long-term results using this particular EIE-technique to the conventional end-to-end (ETE) technique (10) is, however, desirable. Since there seems to be no general consensus of agreement as to what constitutes normal vascular endothelium and since the appearance of endothelial cells varies at different locations of the vascular tree, carefully standardized methods must be used to avoid artifacts and the results must be compared to control materials.

The purpose of the present study was to compare EIE and ETE anastomoses using a surface method (Scanning electron microscopy - SEM). In addition, a histological profile method (light microscopy) was used to study the deeper layers of the vessel walls.

MATERIAL AND METHODS

Rabbits, males and females, weighing between 2.5-4.0 kg, were kept on a standard pellet diet and given water ad libitum.

Eighteen microvascular end-to-end and eighteen end-in-end anastomoses were performed on the central arteries (diameters 0.8-1.2 mm) of the ears.

Operative procedure

The rabbits were anesthetized with intermittent intravenous injections of sodium pentobarbital (Mebumal 60 mg/ml, ACO Läkemedel, Solna, Sweden).

Skin flaps were raised over the central arteries of both ears and the vessels prepared over a distance of 15-20 mm. The exposed arteries were placed in double microvascular clamps and divided. Anastomosis was performed with Dermalon 10-0 (Davis & Geck) using ETE or EIE technique.

Controls

Specimens from 8 central arteries were used as controls. The operative procedures and the preparation of specimens were the same as for all other samples, except that the vessels were not transected and reunited.

End-to-end anastomosis

Superfluous adventitia at both ends of the cut vessel was excised. Three stay sutures were placed 120° apart and 3-5 interrupted sutures placed in between (10). The vascular double clamp was removed and anastomotic patency checked. Finally, the skin incision was sutured.

End-in-end (sleeve) anastomosis

The sleeve technique used was that described by Lauritzen (1978) (8).

SEM and light microscopy of the same vessel segment.

A comparison was performed in 8 vessel segments consisting of 3

ETE anastomoses at 7, 30 and 90 days and 4 EIE anastomoses at 30 and 90 days postoperatively.

Preparation of specimens

Groups, each consisting of three vessels, were taken for processing after intervals of 1-2 hours and 3, 7, 14, 30 and 90 days. A catheter (PA 50-60) was placed in the central artery of the ear 2-3 cm proximal to the anastomosis. Small amounts of Ringer's lactate (composition: Ca^{++} 1.8, K^+ 4.0, Na^+ 151.7, Cl^- 132.5 and $\text{C}_3\text{H}_5\text{O}_3^-$ 26.7 mmol/l) was used to prevent clotting. The vessels were then perfused for 15 minutes at a constant pressure of 120 mm Hg (composition of perfusion fixative: 0.2 M phosphate buffer 400 ml + 50% glutaraldehyde 50 ml + 20% dextran T70 95 ml + dist. H_2O 455 ml) (11). The rabbits were sacrificed at the start of perfusion.

The skin incisions were reopened and the fixed vessel specimens could easily be separated from their surrounding tissue. The vessel segments (20 mm in length) were divided lengthwise using microvascular instruments. One half was processed for SEM, and intimal surface study, the other for light microscopy and longitudinal profile study. The specimens were kept in the same fixative for 12 hours. They were then washed 4-5 times in Millonig's phosphate buffer and kept immersed in the same fluid until SEM processing began.

The vessel specimens were dehydrated in increasing concentrations of alcohol, immersed in freon and dried at the critical point. They were mounted on metal discs with the intima upwards and coated with gold-palladium (200 Å thickness). The specimens were examined in a scanning electron microscope (Stereoscan, Mark II A and a Zeiss Nanolab 2000) and photographed at standard magnifications.

The samples intended for light microscopy were kept in Millonig's phosphate buffer and then dehydrated and imbedded in paraffin (12). Longitudinal 4 μ thick step sections were cut and stained with Weigert-van Gieson stains.

RESULTS

Controls

Normal cells are spindle-shaped with smooth surfaces and with the long cell axis oriented in the direction of blood flow (Fig. 1). Only a few villous projections are seen at the cell borders. At the branching of smaller vessels, the endothelial cells have a more rounded shape (Fig. 2).

The endothelialization of anastomoses

At 1-2 hours

Platelet aggregates, fibrin and erythrocytes were found mainly at the junction between the vessel ends and at sutures in ETE anastomoses and always at the cut end of the telescoped segment in EIE anastomoses. These thrombotic formations showed considerable variations in size and distal protrusion from the anastomosis. Occluding thrombi were seen in one EIE and one ETE anastomosis. Exposed subendothelial structures, at anastomotic and clamp sites, were seen in both types of anastomosis, such areas being covered by a carpet of platelets. Severe luminal narrowing was observed in telescoped vessel segments. Using the EIE technique, pockets were found between the double vessel walls.

At day 3

Using both techniques, denuded areas were found at the anastomotic sites and at the sites of vascular clamping. The borders between undamaged endothelium and exposed subendothelial layers were clearly demarcated. Transformed platelets formed a new intimal surface in areas of endothelial denudation (Figs. 3-5). A few supposedly neo-endothelial cells were seen over damaged areas at this time. Leucocytes were found in large numbers in the anastomosis areas (Fig. 6). The sutures were partly covered by platelets and fibrin. The sleeved vessel ends were covered with platelets, fibrin and blood cells forming small thrombi of varying sizes (Fig. 7-8).

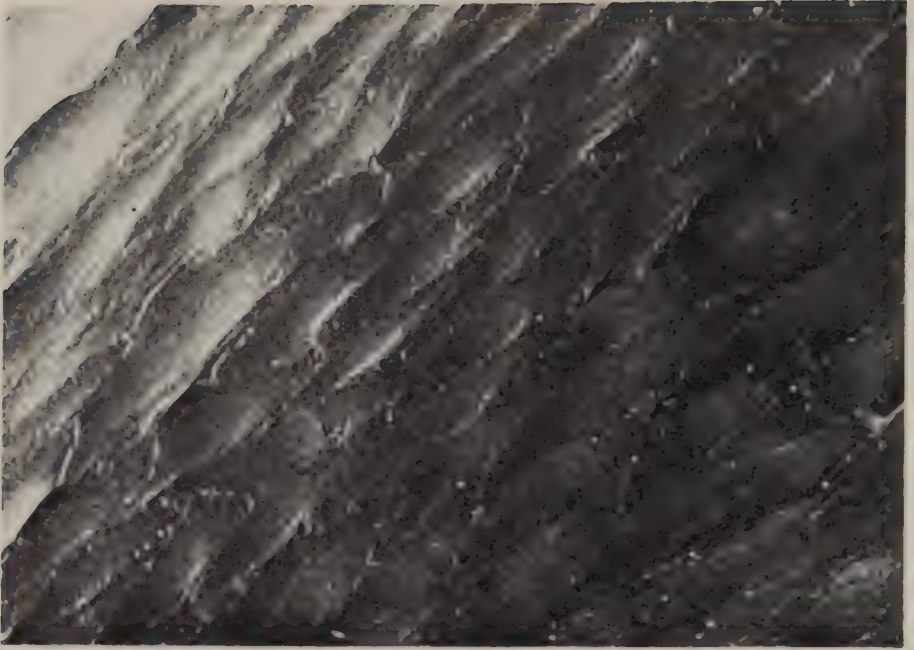


Fig. 1. Control. Intima of the central artery of the rabbit ear. The endothelial cells have a uniform appearance with smooth surfaces and are oriented with the long axis in the direction of blood flow. Perfusion fixation 15 minutes with 120 mm Hg pressure. Ringer-lactate prior to perfusion. SEM x 600.

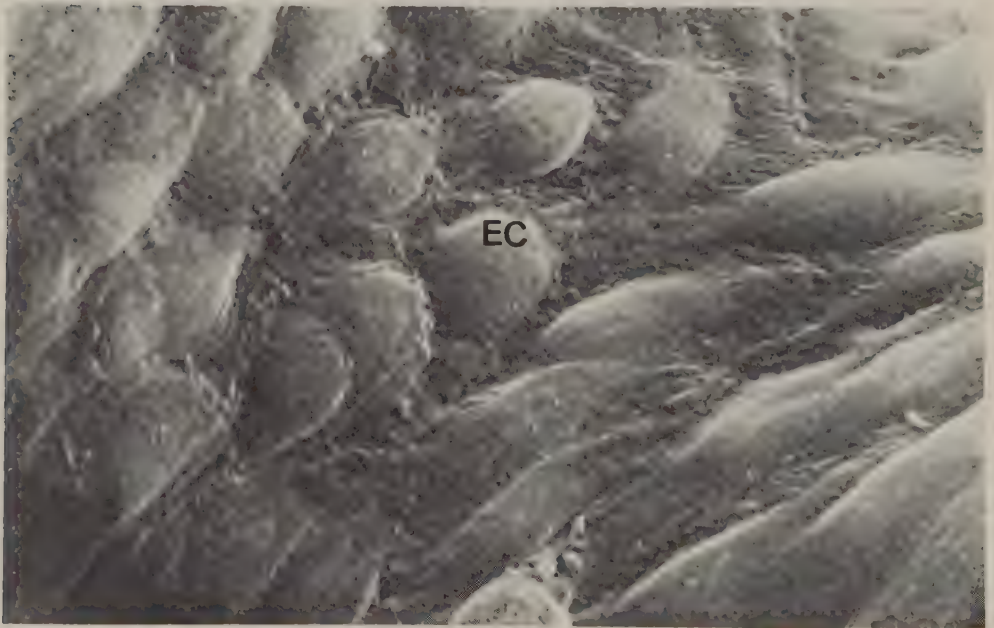


Fig. 2. Control. Intima at the branching of a smaller vessel. The endothelial cells (EC) have a more rounded form with prominent nuclei. To the right spindle-shaped endothelial cells in the main vessel. Perfusion fixation 15 minutes with 120 mm Hg pressure. Ringer-lactate prior to fixation. SEM x 1200.



Fig. 3. ETE anastomosis at day 3. Denuded areas were found at clamp sites. The intima to the left is folded longitudinally. Exposed subendothelium to the right and left is covered with a carpet of platelets. Direction of blood flow ($\blacktriangleright$). SEM x 600.

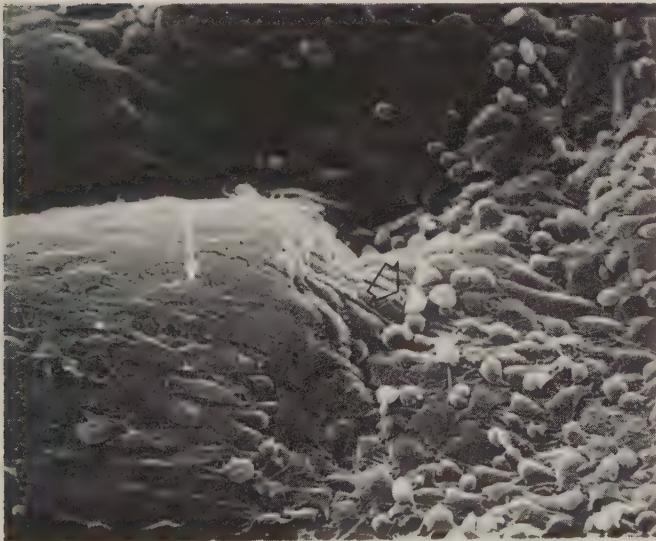


Fig. 4. ETE anastomosis at day 3. Platelets form a "new (temporary) intima". Platelets (white arrow) adhering to the exposed layers are flattened and extended over the surface. Some platelets to the right ($\blacktriangleright$) appear unchanged and loosely attached. SEM x 2400.

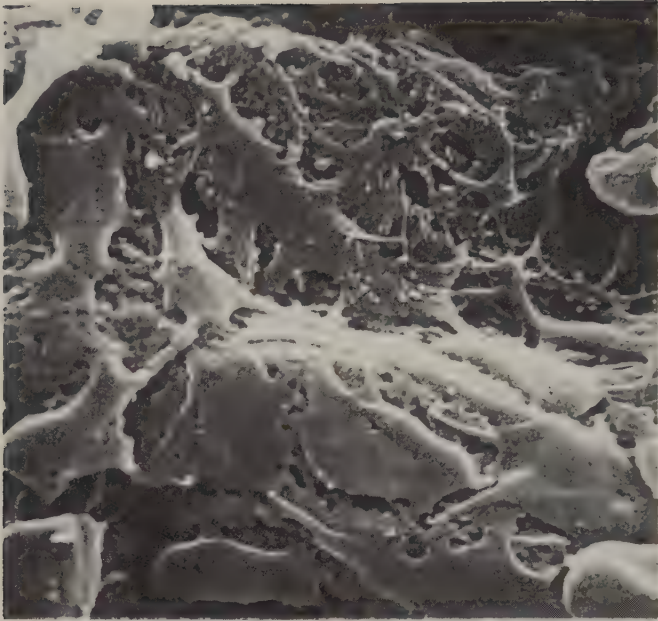


Fig. 5. ETE anastomosis at day 3. The surface seen in Fig. 4 at high magnification. The platelets are flattened and extended over the surface forming a temporary (athrombogenic) intima. SEM x 12000.



Fig. 6. ETE anastomosis at day 3. Close to the anastomosis platelets (P), fibrin (F) and erythrocytes (E) cover the damaged intima. Leucocytes (L) now appear in large numbers. Direction of blood flow (◊). SEM x 2400.



Fig. 7. EIE anastomosis at day 3. In the centre the sleeved vessel end (SV) is covered with a thrombus. Pockets (white arrow) are seen between the telescoped vessel and the external wall. Direction of blood flow ($\blacktriangleright$). SEM x 240.

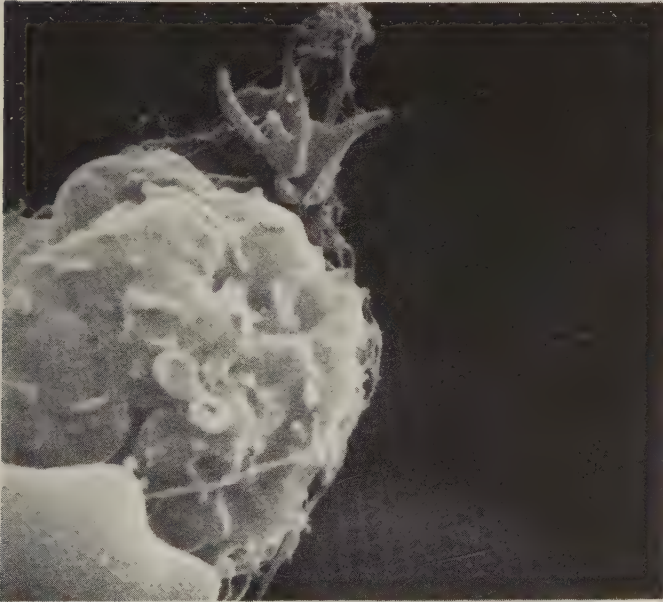


Fig. 8. EIE anastomosis at day 3. The thrombotic material in Fig. 7 seen under high magnification. A platelet aggregate projects intraluminally. A platelet has just been caught and is projecting its pseudopods. Fine fibrin strands are also seen around the platelets. SEM x 12000.

At day 7

Reendothelialization with abnormal endothelial cells was frequently observed at the junction between vessel ends in ETE anastomoses (Figs. 9-10) and at all denuded clamp sites. Endothelialization in EIE anastomoses took place on top of the clot at the telescoped vessel end (Fig. 11). New endothelial cells were frequently continuous with the undamaged endothelial sheet, but isolated cells were also seen in some denuded areas. The neo-endothelium located close to the cut vessel ends consisted of irregularly formed endothelial cells with deep intercellular holes and clefts (Figs. 9, 10). These endothelial cells were seldom oriented in the direction of flow. Minute vessel openings were sometimes observed at anastomotic sites. Thrombosis was found in one ETE and one EIE anastomosis.

At day 14

Following both types of anastomosis, endothelial regeneration was almost complete, but areas where reendothelialization still occurred could be observed in some specimens (Figs. 12-13) .

At day 30

The intimal surface was completely reendothelialized when either of the two anastomotic techniques was used (Fig. 14) . The only exceptions were a few instances with intramural thrombi, which were only partially endothelialized (Fig. 15). The neo-endothelium, however, was frequently abnormal consisting of pleomorphic cells with clefts and holes at cell junctions (Fig. 16).

At day 90

Using both anastomotic techniques, the intraluminal surfaces resembled those at 30 days as seen in Fig. 14. When the telescope technique was used, the intima was still uneven at the junction between the internal and external vessels as seen at 30 days in Fig. 14. An abnormal intima with aberrant endothelial cells was still frequently observed using both anastomotic techniques (Fig. 17).

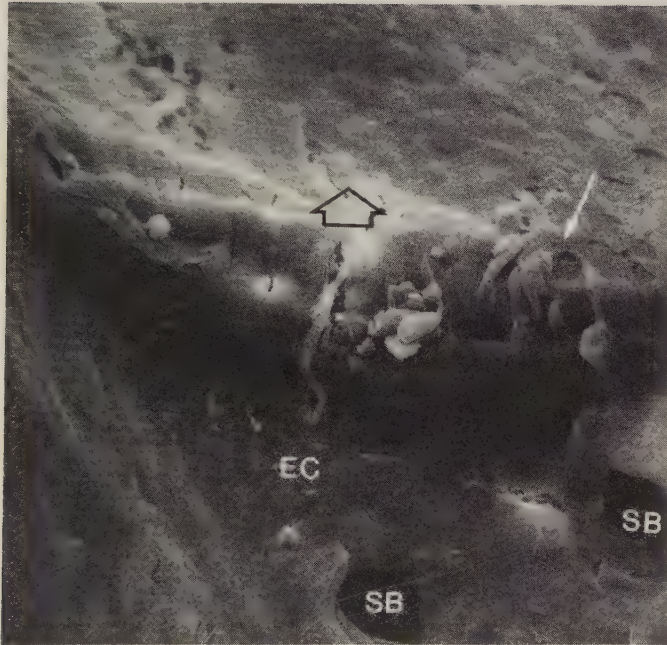


Fig. 9. ETE anastomosis at day 7. The anastomotic site is almost completely reendothelialized. In some areas (white arrow) the endothelial sheet is not complete. Many endothelial cells (EC) have an irregular appearance. Two new side branches (SB) are seen below and to the right. Direction of blood flow (). SEM x 600.

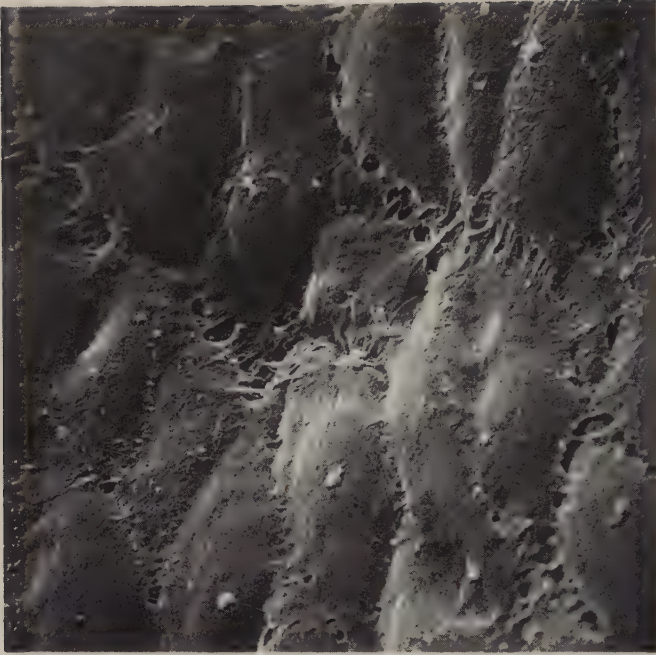


Fig. 10. ETE anastomosis at day 7. The endothelium is composed of cells with irregular forms. Clefts and holes are seen at abnormal intercellular borders. SEM x 2400.

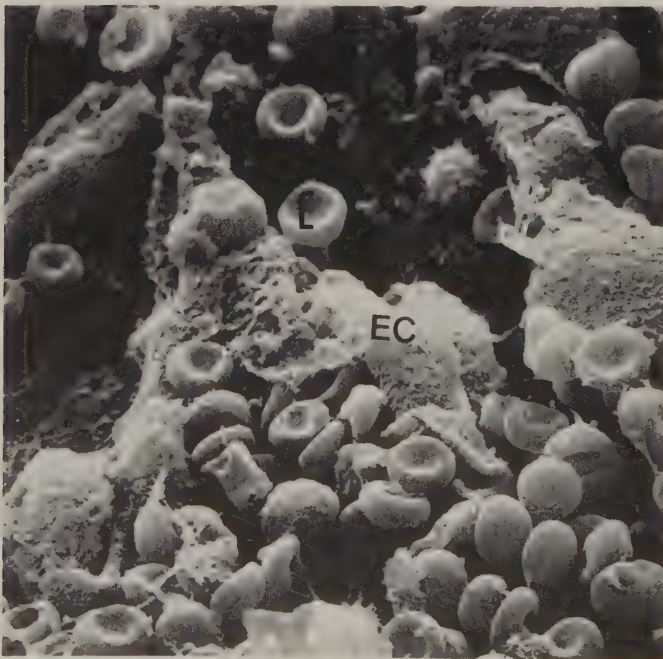


Fig. 11. EIE anastomosis at day 7. Thrombus with many erythrocytes (E) on which scattered new endothelial cells (EC) are seen. SEM x 2400.

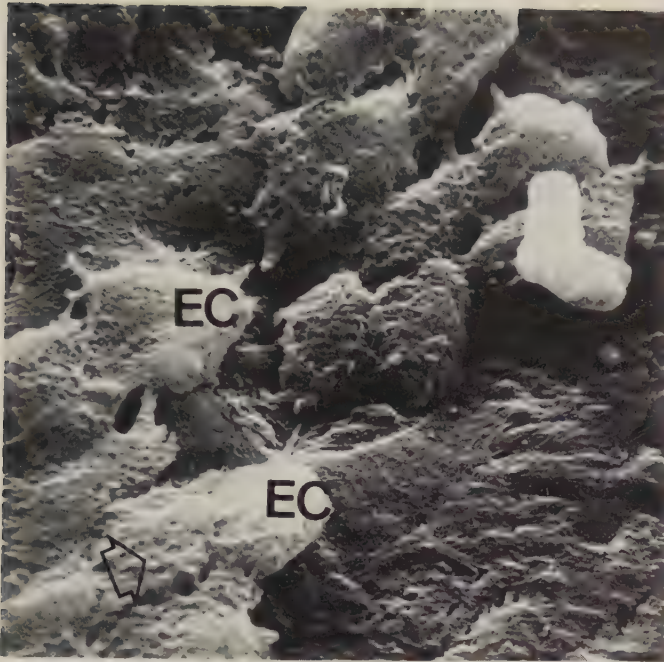


Fig. 12. ETE anastomosis at day 14. Neo-endothelialization is not complete in all areas. Advancing endothelial cells (EC) are closing remaining defects. Direction of blood flow (). SEM x 2400.

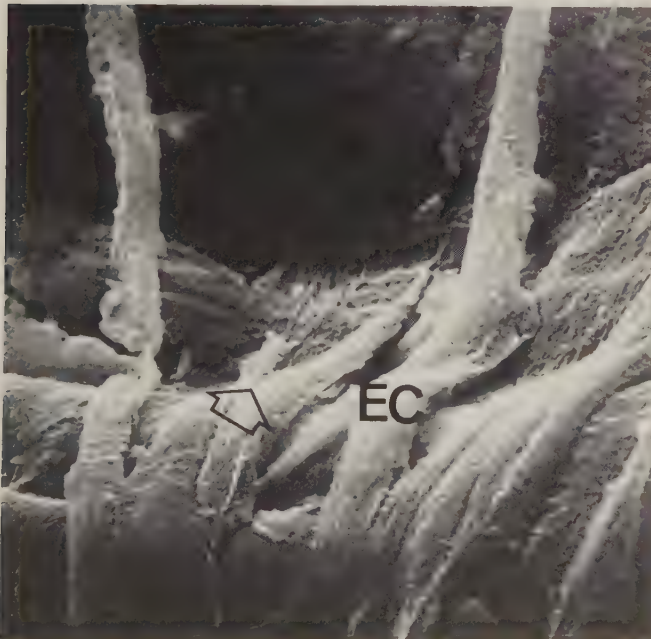


Fig. 13. ETE anastomosis at day 14. Endothelial cells (EC) are bridging defects at the anastomotic line. Direction of blood flow (). SEM x 2400.

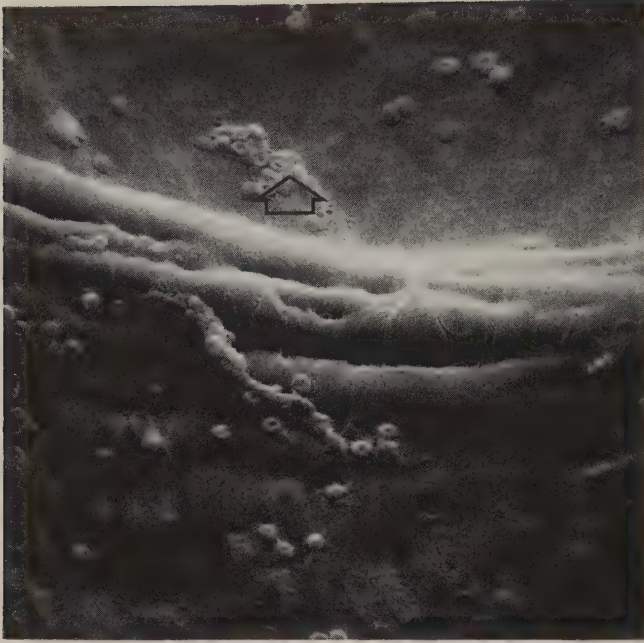


Fig. 14. EIE anastomosis at day 30. Reendothelialization is complete at the sleeved vessel end. The intima is uneven at the junction between the vessels. Direction of blood flow (◻). SEM x 600.

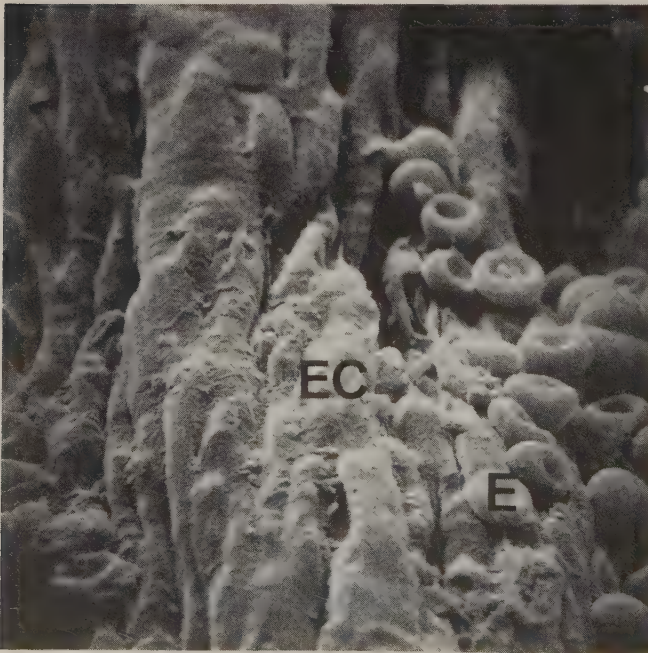


Fig. 15. EIE anastomosis at day 30. Intramural thrombotic material was almost covered by endothelium (EC). Erythrocytes (E). SEM x 2400.

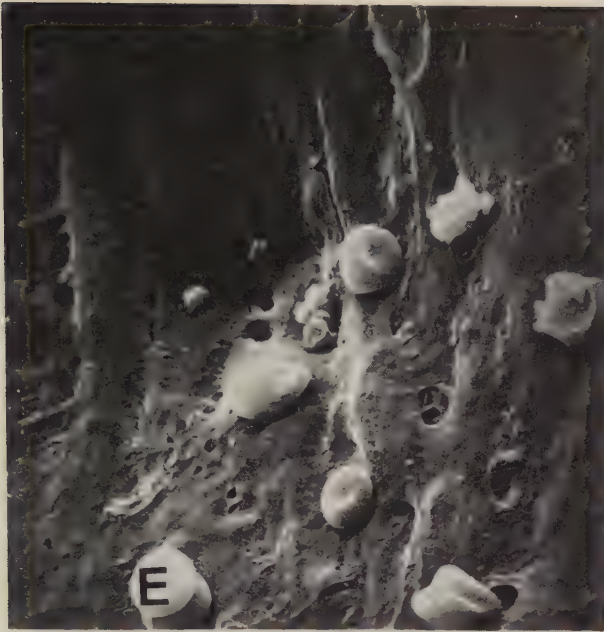


Fig. 16. EIE anastomosis at day 30. The neo-endothelium consists of abnormal-looking cells and many holes at the intercellular borders. Erythrocytes (E). SEM x 2400.



Fig. 17. ETE anastomosis at day 90. Abnormal endothelium with irregular cells of different sizes and shapes. The intercellular borders are also abnormal with frequently occurring holes and large defects. The unknown cell in the centre (■) seems to creep out from deeper layers. SEM x 2400.

L

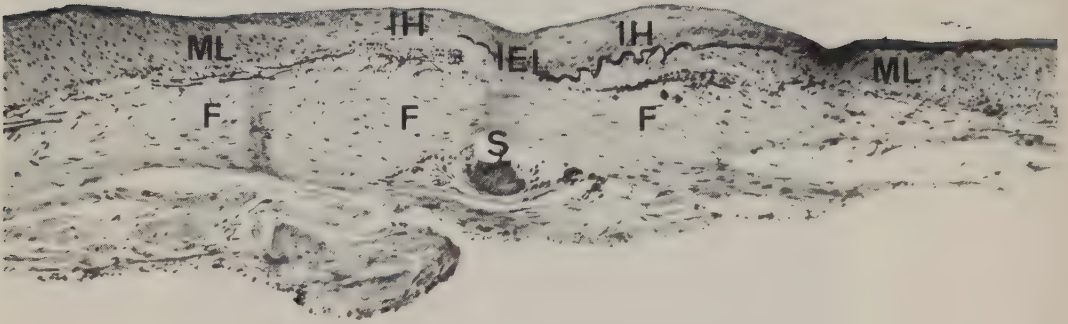


Fig. 18. ETE anastomosis at 90 days. The intima consists of multilayer hyperplasia (IH). The internal elastic lamina (IEL) is fragmented and the external wall is fibrotic (F). Normal medial layer (ML). Suture (S). Lumen (L). Weigert-van Gieson Stain. Profile as seen in light microscopy. 135 x.

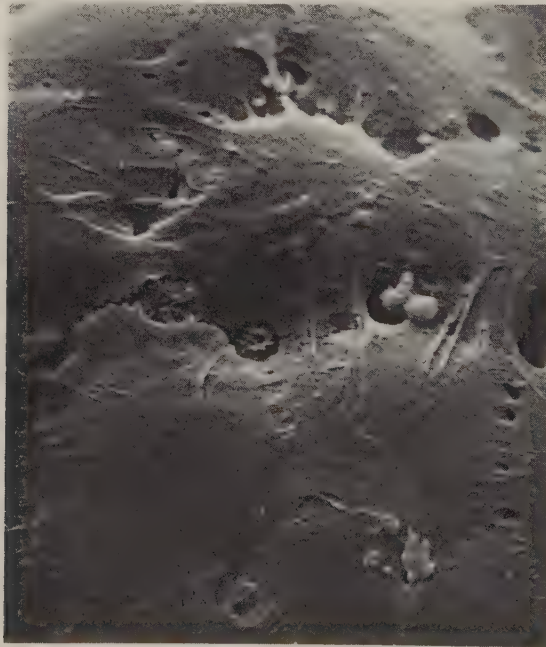


Fig. 19. The abnormal endothelium of the vessel segment seen in Fig. 18. The endothelial cells are irregular in form and size with abnormal intercellular bridges and deep holes and clefts at cell junctions. SEM. 2400 x.

Comparison between SEM and light microscopy findings in the same vessel segment (Figs. 18-19)

In 7 vessels (3 ETE/4 EIE) an abnormal endothelium (SEM) was covering areas of intimal hyperplasia (light microscopy) up to 90 days postoperatively. In 1 EIE anastomosis (at 90 days) with evident intimal hyperplasia the endothelial sheet was uneven, but the cells looked almost normal.

DISCUSSION

There is still considerable controversy as to the best way to process specimens for SEM. Many recently described methods in which vessels are rinsed and perfused prior to fixation have been found to cause endothelial damage (14). Perfusion fixation under physiological pressure seems to cause few artifacts (14). In this work we have tried to further refine a technique so as to cause a minimum of endothelial damage. Using different electrolytic solutions (Wieslander, to be published), our findings substantiate a previous report by Ackland (13): Ringer lactate seems to cause the least number of artifacts.

The first specimens in this study were taken at 1-2 hours post-anastomosis. We have previously studied the accumulation of isotope labelled platelets occurring immediately after releasing the vascular clamps (15). Using the ETE technique, a rapid and pronounced accumulation of platelets was registered with a maximum reached within the first 30 minutes, followed by a gradual decrease during the next 30-60 minutes to a steady state level. The accumulation of isotope labelled platelets following the EIE technique was considerably less, and the steady state level was lower. SEM findings at 1-2 hours show the state of the intima after the major platelet aggregates at the transected vessel ends, sutures and suture holes have dissolved (16,17). The platelets which remain seal the anastomotic defect and the suture holes. Exposed subendothelial layers and sutures were covered by activated platelets constituting an athrombogenic carpet: this

seems to be a prerequisite for subsequent endothelialization. The results indicate that sutures (polyamide) and suture holes account for a high platelet accumulation during the first postoperative hour. The reason for the modest platelet accumulation at the sleeved vessel end immediately postoperatively is not known. One reason might be the high flow velocities in the narrow sleeved segment, another that there are no sutures or suture holes. However, Sully et al. (1982) (18) found a progressive build-up of thrombotic material between the internal and external sleeves during the first postoperative week, when they compared the ETE technique with their modification of the EIE technique introduced by Meier 1978 (19). The observations in our study of a thrombus at the sleeved vessel end at days 3 and 7 (Fig. 7 and 11) might also mean an increased risk of late anastomotic failures using the sleeve technique in small vessels. Several studies have shown a slight patency decrease following the use of EIE technique as compared to the ETE technique (12,18, 20). In our studies, in spite of the severe luminal narrowing and inevitably turbulent blood flow, the number of total occlusions was the same for both techniques.

No obvious difference between the two techniques was seen regarding the time required for reendothelialization, which took at least two weeks. Per continuitatem regeneration of the endothelium began at undamaged endothelial borders (21) using both techniques, but in many instances isolated endothelial cells were also found in denuded areas. These endothelial cells could either be derived from the blood (22,23) or originate from deeper layers in the vessel wall (24). Our findings of single endothelial cells on top of thrombi at the ends of sleeved vessels support a blood-born genesis of endothelial cells.

Abnormal neo-endothelium with pleomorphic cells separated by irregular bridges, clefts and holes were found in 7 of 8 vessel segments with intimal hyperplasia. Little is known about the correlation of these two findings. In a previous study (Wieslander and Rausing, 1983) (12) intimal hyperplasia was a constant finding in the repair process using either EIE or ETE technique. However, the process was proliferating only up to 30 days, see-

ming then to become stagnant or even to condensate. One hypothesis regarding intimal hyperplasia is that the turbulent blood flow following vascular surgery causes continuous damage to endothelial cells inducing deposition of activated platelets and the release of thromboxane A_2 . This concurs with a theory regarding intimal hyperplasia, namely, that the proliferation of neointimal cells occurs in order to increase the production of the antiaggregatory prostaglandin PGI_2 , and hence counteract a high local release of thromboxane A_2 , endangering vessel patency (25). The regulatory mechanisms governing platelet adhesion, aggregation, disaggregation, normal and abnormal endothelialization and the development of intimal hyperplasia need further elaborate studies (26). Such studies should also consider the local role of prostaglandins and the fibrinolytic activity following microvascular anastomosis.

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